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REMARKS

Status of the Claims

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

In the specification, paragraphs have been amended on pages 106, 108, 110, and 112.

Claims 6, 33, 36 and 37 are currently being amended.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

Exemplary support for “phosphatase activity” in claims 6 and 33 is found in the specification on page 25, lines 20-22. Support for “catalytic domain” in claims 36 and 37 is found in the specification on page 10, lines 9-12.

Upon entry of this Amendment, claims 6 and 33-37 will remain pending in the application.

Issues Related to the Specification

The Examiner objects to the Specification because it is unclear what “FLv” stands for in Tables 1-4. Applicants note that in Tables 1-4, the term “FLv” should be replaced with the term “FL.” As stated in the description of Table 1 on page 105, lines 15-16, “‘FL/Cat’ refers to the length of the gene, with ‘FL’ indicating full length and ‘Cat’ indicating that only the catalytic domain is present.” Additionally, the descriptions of Tables 2-4 (see page 107, line 7, page 109, line 5, page 111, line 8) state “...are as described above for Table 1.” Applicants have amended Tables 1-4 to state “FL” instead of “FLv.”

Claim Objections

The Examiner objects to claim 6 because it recites “(a)”, but absent “(b)” “(a)” is not needed. The Examiner also states that “TAK1” and “Ras” in claim 6 should be written out in full, followed by the abbreviation in parenthesis. Applicants have amended claim 6 to delete the term “(a).” Additionally, Applicants have deleted the terms “TAK1” and “Ras” from claim 6.

The Examiner advises that should claim 34 be found allowable, claim 35 will be objected to under 37 C.F.R. § 1.75 as being a substantial duplicate thereof. Applicants take note of the Examiner’s statement.

Claim Rejections - 35 U.S.C. § 101

Claims 6 and 33-37 are rejected by the Examiner under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific and substantial asserted utility or a well established utility. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner notes Applicants’ classification of SEQ ID NO: 8 as a serine threonine phosphatase and as “PP2C” phosphatase via their comparison of SEQ ID NO: 8 to a mouse putative PP2C sequence, namely GenBank GI 12850332. The Examiner states that GenBank GI 12850332 was replaced by a newer version (GI 26378394) that defines the polypeptide as an unnamed protein product. The Examiner asserts that a sequence search performed at the PTO did not reveal any homology between SEQ ID NO: 8 and a protein for which PP2C activity was demonstrated.

In Applicants’ August 21, 2003 Amendment, Applicants submitted an “NCBI Conserved Domain Search” analysis, which classifies the amino acid sequence of SEQ ID NO:8 as a *bona fide* PP2C serine/threonine phosphatase, and shows the “significant alignment” of SEQ ID NO:8 to other serine/threonine phosphatases. The Examiner asserted on page 14 of the outstanding Office Action that the NCBI Conserved Domain Search analysis was not persuasive because Applicants did not disclose the actual alignment, overall percent identity with the given sequences and whether they represent actual PP2Cs. In

response to the Examiner's assertion, Applicants submit, as Exhibit 1, the attached NCBI Conserved Domain Search analysis which shows the alignment of SEQ ID NO:8 with sequences from the database, the overall percent identity between SEQ ID NO:8 and sequences from the database and that many of the sequences identified in the analysis are PP2Cs. Therefore, the amino acid sequence of SEQ ID NO:8 as a *bona fide* PP2C serine/threonine phosphatase.

The Examiner further asserts that even accepting the plausible utility of the amino acid sequence of SEQ ID NO:8 being a PP2C, since there are at least six distinct PP2C gene products, one of ordinary skill in the art would not know which type of PP2C the present invention is directed to. Applicants respectfully disagree with the Examiner. Amended claims 6, 33, 36 and 37 and previously presented claims 34-35 recite the necessary structure or structure and function of the amino acid sequence so that a person of ordinary skill in the art would know which type of PP2C the present invention is directed to.

Finally, the Examiner asserts that the specification does not disclose a specific function of the polypeptide of SEQ ID NO:8, its relationship to any disease, or any specific real world use. The Examiner asserts that it appears that the main utility of the polypeptide of SEQ ID NO:8 is to carry out further research. Applicants respectfully disagree with the Examiner.

As discussed above and as evidenced by the attached NCBI Conserved Domain Search analysis (Exhibit 1), the polypeptide of the present invention is a *bona fide* PP2C polypeptide. The specification is replete with examples of the utility of such polypeptides and their relationship to diseases. *See* pages 16-19 of Applicants' August 21, 2003 Amendment. For example, at page 64, lines 21-26 the specification states:

"treatment may be effectuated to a wide range of diseases, including cancer, pathophysiological hypoxia, cardiovascular disorders, Papillon-Lefevre syndrome, Cowden disease, ectodermal dysplasia, Moebius syndrome, Bjornstad syndrome, Bannayan Zonana syndrome, schizophrenia and hamartomas. Of particular importance is treatment to various type of cancers. Accordingly, the present invention provides methods for treating pathologies, including breast cancer,

urogenital cancer, prostate cancer, head and neck cancer, lung cancer, synovial sarcomas, renal cell carcinoma, non-small cell lung cancer, hepatocellular carcinoma, pancreatic endocrine tumors, stomach cancer, glioblastoma, colorectal cancer, and thyroid cancer."

Furthermore, as described in the attached articles (Exhibits 2 and 3), there is an established relationship between PP2C and diseases, such as cancer. For example, Sanli et al. show a relationship between PP2C expression and apoptosis in breast cancer cells.¹ Additionally, Kitamura et al. show a relationship between PP2C expression and hepatocarcinogenesis.²

For the reasons discussed above, the present invention is supported by both a specific and substantial asserted utility and a well established utility.

Claim Rejections - 35 U.S.C. § 112, First Paragraph

A. Rejection of claims 6 and 33-37 for allegedly failing to be supported by a specific and substantial utility or a well established utility.

Claims 6 and 33-37 are rejected by the Examiner under 35 U.S.C. § 112, first paragraph for failing to be supported by a specific and substantial utility or a well established utility. As discussed above with respect to the Examiner's rejection under 35 U.S.C. § 101, claims 6 and 33-37 are supported by a specific and substantial utility and a well established utility. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejection.

B. Rejection of claims 6 and 33 for alleged lack of written description

Claims 6 and 33 are rejected by the Examiner under 35 U.S.C. § 112, first paragraph for lack of written description. Applicants respectfully request reconsideration and withdrawal of the rejection.

¹ Sanli et al. *Oncology Reports* 10:2083-2088 (2003).

² Kitamura et al. *Jpn. J. Cancer Res.* 83:66-71 (1992).

The Examiner asserts that the phrase “wherein said polypeptide is involved in at least one of integrin signal transduction, the TAK1 signaling pathway, a cellular channel, a cyclin dependent kinase, and the Ras pathway” does not define the function of the polypeptide because “involvement” in a process does not define the function. Applicants respectfully disagree. However, in order to expedite prosecution, Applicants have deleted this phrase from claim 6.

The Examiner asserts that claim 33 encompasses many functionally unrelated polypeptides. Applicants respectfully disagree. However, in order to expedite prosecution, Applicants have amended claim 33 to recite that the polypeptide has phosphatase activity. Additionally, Applicants have amended claim 33 so that the polypeptide does not lack the catalytic domain.

C. Rejection of claims 6 and 33 for alleged lack of enablement

The Examiner asserts that the specification does not reasonably provide enablement for a polypeptide having an amino acid sequence that is 90% identical to SEQ ID NO:8 that is involved in various process and has an undefined activity. Applicants have amended claims 6 and 33 to recite that the polypeptide has phosphatase activity. Applicants have also amended claim 33 so that the polypeptide retains the catalytic domain. Page 25, lines 20-22 of the specification state that “[p]hosphatase activity is preferably the interaction with a natural binding partner followed by removal of a phosphate from a phosphorylated substrate.” A person of ordinary skill in the art could measure the phosphatase activity of the polypeptides using techniques well known in the art without undue experimentation in order to identify the claimed polypeptides. Assays involving measurement of phosphatase activity are described throughout the specification. *See, e.g.*, page 24, line 25, through page 25, line 4.

Claim Rejections - 35 U.S.C. § 112, Second Paragraph

Claims 6, 33, 36 and 37 are rejected by the Examiner under 35 U.S.C. § 112, second paragraph as being allegedly indefinite. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Examiner asserts that in claim 6, “involvement” in any process does not define the function. Applicants do not agree with the Examiner. However, in order to expedite prosecution, Applicants have deleted the term “involvement” from claim 6.

The Examiner asserts that claim 33 recites “N terminal domain, a C terminal catalytic domain, a catalytic domain, a C terminal domain...” and that this phrase is indefinite because it is unclear what is the difference among these domains. Applicants respectfully disagree. The domains recited in claim 33 are described in detail in the specification on page 9, line 22, through page 12, line 22. The specification provides a description of the function of the domains (*See, e.g.*, page 9, lines 23-27), the size of the domains (*See, e.g.*, page 10, lines 10-11), and techniques that a person of ordinary skill in the art can use to identify the domains (*See, e.g.*, page 11, lines 9-12). Therefore, a person of ordinary skill in the art would know which fragments of SEQ ID NO:8 are encompassed by claim 33.

Finally, the Examiner asserts that in claims 36 and 37, it is unclear whether any other phosphatase activity in addition to the protein phosphatase 2C activity is implied for fragments. Applicants have amended claims 36 and 37 to recite that the fragment comprises a catalytic domain. Support is found on page 10, lines 9-12 and page 25, lines 20-22.

Claim Rejections - 35 U.S.C. § 103

Claims 6 and 33 are rejected by the Examiner under 35 U.S.C. § 103 as being obvious over Hillier et al. The Examiner asserts that Hillier et al. teach an mRNA that is similar to PP2C and that it would have been obvious to use this EST to produce the encoded PP2C polypeptide. Applicants respectfully request reconsideration and withdrawal of the rejection.

In order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references (or references when combined) must teach or suggest all the claim limitations. See MPEP 2142. The Examiner has failed to establish a *prima facie* case of obviousness because all three of the criteria have not been met.

The Examiner asserts that one of ordinary skill in the art would have been motivated to produce the encoded human protein in order to produce an antibody against it. However, in addition to there being no suggestion or motivation in Hillier for a person of ordinary skill in the art to transcribe and translate the described cDNA clone so as to produce a PP2C serine/threonine phosphatase protein, also, there is no suggestion or motivation in Hillier et al. for person of ordinary skill in the art to produce an antibody against the encoded protein.

Moreover, the Court of Appeals for the Federal Circuit has found that a cDNA is not obvious even where the complete amino acid sequence for a protein is known. *See, e.g., In Re Bell*, 991 F2d 781 (CAFC 1993). Therefore, it cannot be the case that a novel protein sequence is rendered obvious because a much shorter EST exists in the prior art.

The Examiner also asserts that one of ordinary skill in the art would have a reasonable expectation of success because the mRNA was defined as “similar to PP2C.” At best, the Examiner is using an improper “obvious to try” standard. However, “‘obvious to try’ has long been held to not constitute obviousness.” *In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210 (Fed. Cir. 1995).

With respect to claim 6, the Examiner asserts that Hillier et al. renders claim 6 obvious because claim 6 does not require the sequence of the claimed polypeptide to be 90% identical over the entire SEQ ID NO:8. Applicants note that SEQ ID NO:8 comprises 459 amino acids. As discussed above, a person of ordinary skill in the art would not have been motivated to transcribe and translate the described cDNA clone so as to produce a PP2C serine/threonine phosphatase protein. However, assuming *arguendo* that the cDNA clone of Hillier et al. was transcribed and translated, the resulting amino acid sequence would be about 182 amino acids (the cDNA clone of Hillier et al. is 547 bp). Applicants note that an amino acid sequence that is 182 amino acids can not be 90% identical to an amino acid sequence which has 459 amino acids.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date January 28, 2004

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results of **BLAST**

BLASTP 2.2.6 [Apr-09-2003]

Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1072110441-10830-135706414391.BLASTQ4

Query=

(459 letters)

Database: All non-redundant GenBank CDS

translations+PDB+SwissProt+PIR+PRF

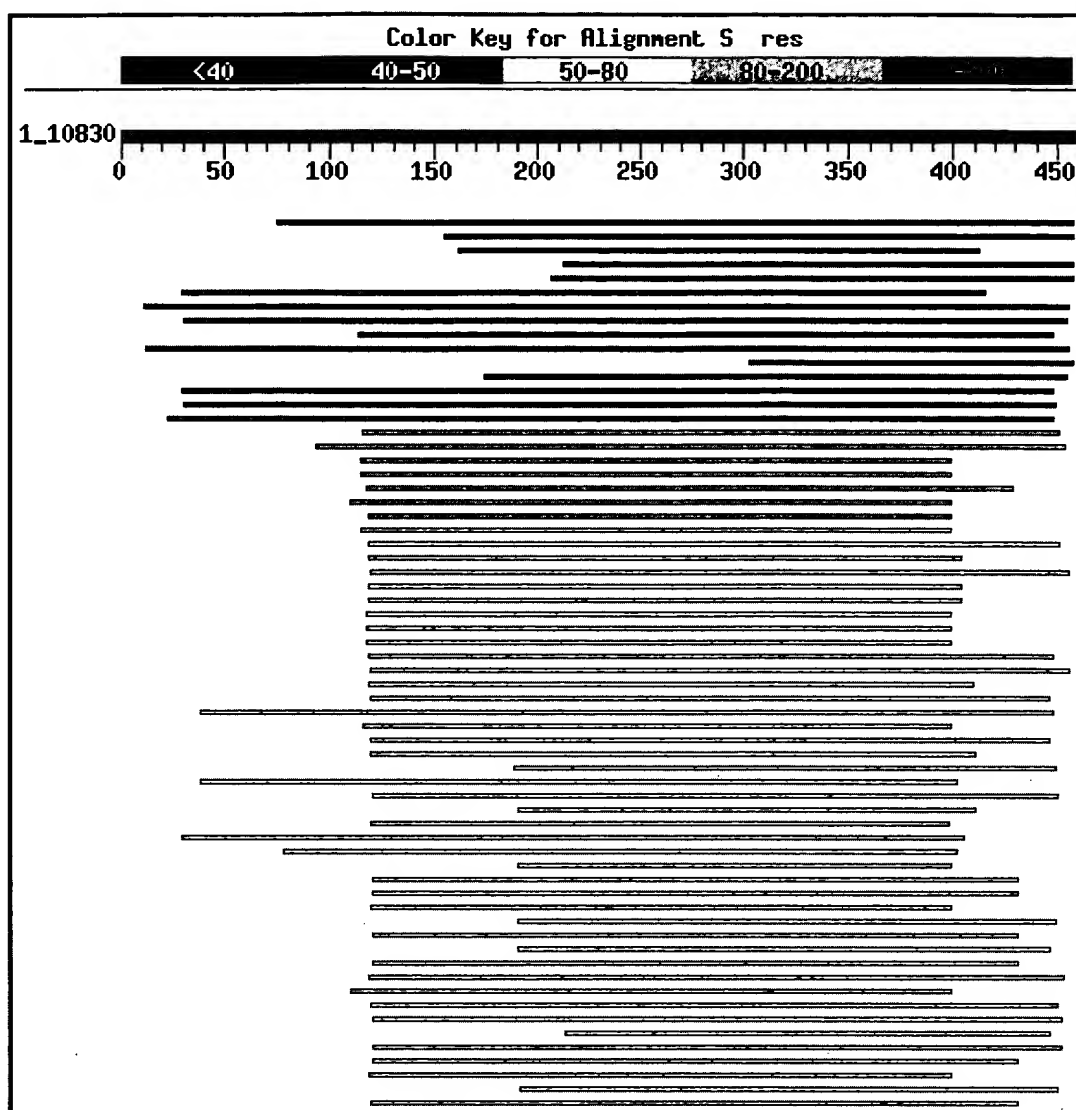
1,581,064 sequences; 518,058,112 total letters

If you have any problems or questions with the results of this search please refer to the [BLAST FAQs](#)

[Taxonomy reports](#)

Distribution of 100 Blast Hits on the Query Sequence

Mouse-over to show defline and scores. Click to show alignments



Sequences producing significant alignments:			Score (bits)	E Value	
gi 39573724 ref NP_945149.1 	protein phosphatase 2C eta iso...	704	0.0	L	
gi 26338027 dbj BAC32699.1 	unnamed protein product [Mus mu...	544	e-153	L	
gi 21389471 ref NP_653242.1 	hypothetical protein FLJ32332 ...	512	e-144	L	
gi 16307111 gb AAH09644.1 	FLJ32332 protein [Homo sapiens]	499	e-140	L	
gi 12856386 dbj BAB30649.1 	unnamed protein product [Mus mu...	445	e-124	L	
gi 28892713 ref NP_795893.1 	RIKEN cDNA A430075L18 gene [Mu...	357	2e-97	L	
gi 29611652 ref NP_082258.1 	protein phosphatase 2a, cataly...	335	1e-90	L	
gi 33873319 gb AAH11803.2 	MGC19531 protein [Homo sapiens]	334	2e-90	L	
gi 31127152 gb AAH52910.1 	Unknown (protein for IMAGE:30075...	332	1e-89		
gi 34859725 ref XP_215663.2 	similar to protein phosphatase...	327	3e-88	L	
gi 34526234 dbj BAC85206.1 	unnamed protein product [Homo s...	311	2e-83		
gi 39930533 ref NP_005158.4 	hypothetical protein MGC19531 ...	271	3e-71	L	
gi 17539110 ref NP_502329.1 	protein phosphatase 2C-like (4...	261	2e-68	L	

gi 31203861 ref XP_310879.1	ENSANGP00000008051 [Anopheles ...	256	6e-67	
gi 19920426 ref NP_608468.1	CG17598-PA [Drosophila melanog...	255	1e-66	L
gi 3242077 emb CAA05875.1	protein phosphatase 2C [Arabidop...	89	1e-16	
gi 7768151 emb CAB90633.1	protein phpsphatase 2C (PP2C) [F...	88	3e-16	
gi 30685388 ref NP_173199.2	protein phosphatase 2C (PP2C) ...	88	3e-16	
gi 26452332 dbj BAC43252.1	unknown protein [Arabidopsis th...	88	3e-16	
gi 33309516 gb AAQ03211.1	protein phosphatase 2C [Prunus a...	86	2e-15	
gi 21902025 dbj BAC05575.1	protein phosphatase 2C-like pro...	81	3e-14	
gi 15222398 ref NP_172223.1	protein phosphatase 2C (PP2C),...	80	7e-14	
gi 8778461 gb AAF79469.1	F1L3.26 [Arabidopsis thaliana]	79	1e-13	
gi 3643082 gb AAC36697.1	protein phosphatase-2C; PP2C [Mes...	79	2e-13	
gi 15229745 ref NP_187748.1	protein phosphatase 2C (PP2C),...	79	2e-13	
gi 609658 gb AAA67321.1	protein phosphatase 2C (ptc3+)	77	7e-13	
gi 16226419 gb AAL16163.1	AT5g59220/mnc17_110 [Arabidopsis...	77	1e-12	
gi 15237839 ref NP_200730.1	protein phosphatase 2C (PP2C),...	77	1e-12	
gi 15236110 ref NP_194338.1	protein phosphatase ABI1 [Arab...	76	1e-12	
gi 509419 emb CAA55484.1	ABI1 [Arabidopsis thaliana]	76	1e-12	
gi 10432446 emb CAC10358.1	protein phosphatase 2C [Nicotia...	76	1e-12	
gi 14334800 gb AAK59578.1	putative protein phosphatase ABI...	76	1e-12	
gi 19113999 ref NP_593087.1	protein phosphatase 2c homolog...	76	1e-12	
gi 15227078 ref NP_180499.1	protein phosphatase 2C (PP2C),...	75	2e-12	
gi 38109901 gb EAA55700.1	hypothetical protein MG01351.4 [...	75	2e-12	
gi 15242022 ref NP_200515.1	protein phosphatase 2C, ABI2 [...	75	3e-12	
gi 32406686 ref XP_323956.1	hypothetical protein [Neurospo...	75	3e-12	
gi 25352225 pir F86206	hypothetical protein [imported] - A...	75	4e-12	
gi 17555234 ref NP_497949.1	protein phosphatase 2C, possib...	74	4e-12	L
gi 4336436 gb AAD17805.1	protein phosphatase type 2C [Lotu...	71	4e-11	
gi 32492311 emb CAE03844.1	OSJNBb0089K06.2 [Oryza sativa (...	70	9e-11	
gi 39591591 emb CAE71168.1	Hypothetical protein CBG18025 [...	69	1e-10	
gi 6478146 emb CAB61839.1	putative serine/threonine phosph...	69	1e-10	
gi 15220548 ref NP_176948.1	protein phosphatase 2C (PP2C),...	69	2e-10	
gi 34908256 ref NP_915475.1	putative protein phosphatase 2...	68	4e-10	
gi 30693757 ref NP_175057.2	protein phosphatase 2C (PP2C),...	67	8e-10	
gi 34902050 ref NP_912371.1	putative Serine/threonine phos...	67	1e-09	
gi 26331022 dbj BAC29241.1	unnamed protein product [Mus mu...	66	1e-09	L
gi 34857062 ref XP_227247.2	similar to protein phosphatase...	66	1e-09	L
gi 31197933 ref XP_307914.1	ENSANGP00000021879 [Anopheles ...	65	2e-09	
gi 18417190 ref NP_567808.1	protein phosphatase 2C (PP2C),...	65	3e-09	
gi 29568126 gb AAO43055.1	protein phosphatase 2C epsilon [...	65	3e-09	L
gi 18395099 ref NP_564165.1	protein phosphatase 2C (PP2C),...	65	3e-09	
gi 30689144 ref NP_194903.2	protein phosphatase 2C (PP2C),...	64	6e-09	
gi 15242182 ref NP_199989.1	protein phosphatase 2C (PP2C),...	64	9e-09	
gi 15225656 ref NP_181547.1	protein phosphatase 2C (PP2C),...	63	9e-09	
gi 7487243 pir T00750	probable protein phosphatase 2C [imp...	63	1e-08	
gi 19387276 gb AAL87187.1	putative protein phosphatase 2C ...	63	1e-08	
gi 21537096 gb AAM61437.1	protein phosphatase type 2C, put...	62	2e-08	
gi 7486331 pir T05095	hypothetical protein F28M20.60 - Ara...	62	3e-08	
gi 38345197 emb CAE02890.2	OSJNBa0015K02.7 [Oryza sativa (...	62	3e-08	
gi 32488822 emb CAE03557.1	OSJNBa0085I10.2 [Oryza sativa (...	61	4e-08	
gi 18399423 ref NP_565480.1	protein phosphatase 2C (PP2C),...	60	7e-08	
gi 31220270 ref XP_316899.1	ENSANGP00000011103 [Anopheles ...	60	8e-08	
gi 19920896 ref NP_609154.1	CG7115-PB [Drosophila melanoga...	60	8e-08	L
gi 34913678 ref NP_918186.1	OSJNBa0062A24.10 [Oryza sativa...	60	1e-07	
gi 28393269 gb AAO42063.1	putative protein phosphatase 2C ...	59	1e-07	
gi 31216428 ref XP_316230.1	ENSANGP00000017684 [Anopheles ...	59	2e-07	
gi 29248941 gb EAA40463.1	GLP_159_22507_21425 [Giardia lam...	59	2e-07	
gi 26390163 dbj BAC25853.1	unnamed protein product [Mus mu...	58	4e-07	L
gi 15236548 ref NP_194914.1	protein phosphatase 2C (PP2C),...	58	5e-07	

gi 21539551 gb AAM53328.1	putative protein phosphatase typ...	58	5e-07	
gi 38105092 gb EAA51559.1	hypothetical protein MG03154.4 [...	57	7e-07	
gi 2114221 dbj BAA19990.1	phosphatase 2C motif [Homo sapiens]	57	8e-07	L
gi 18086420 gb AAL57666.1	AT5g53140/MFH8_8 [Arabidopsis th...	56	1e-06	
gi 7661862 ref NP_055449.1	protein phosphatase 1F; partner...	56	1e-06	L
gi 20146110 dbj BAB88944.1	protein phosphatase 2C [Mesembr...	56	2e-06	
gi 23506609 gb AAN37903.1	putative serine/threonine phosph...	56	2e-06	
gi 228508 prf 1805227A	protein phosphatase 2C	55	2e-06	
gi 25352221 pir T52337	phosphoprotein phosphatase (EC 3.1....	55	2e-06	
gi 15230495 ref NP_190715.1	protein phosphatase 2C (PP2C),...	55	2e-06	
gi 6320198 ref NP_010278.1	serine-threonine protein phosph...	55	2e-06	
gi 2137765 pir I49016	phosphoprotein phosphatase (EC 3.1.3...	55	3e-06	L
gi 6319415 ref NP_009497.1	protein phosphatase type 2C; Pt...	55	3e-06	
gi 2137695 pir S65672	phosphoprotein phosphatase (EC 3.1.3...	55	3e-06	L
gi 33859600 ref NP_035281.1	protein phosphatase 1B, magnes...	55	3e-06	L
gi 27806079 ref NP_776855.1	protein phosphatase 1B (former...	55	3e-06	L
gi 18423476 ref NP_568786.1	protein phosphatase 2C (PP2C),...	55	3e-06	
gi 30684431 ref NP_188351.2	protein phosphatase 2C (PP2C) ...	55	4e-06	
gi 25144464 ref NP_741087.1	protein phosphatase type-2C (5...	54	4e-06	L
gi 12666519 emb CAC28024.1	protein phosphatase 1B2 53 kDa ...	54	4e-06	L
gi 16416087 emb CAB91227.2	related to Type 2C Protein Phos...	54	5e-06	
gi 15081703 gb AAK82506.1	At1g78200/T11I11_14 [Arabidopsis...	54	5e-06	
gi 32415453 ref XP_328206.1	Type 2C Protein Phosphatase re...	54	5e-06	
gi 4505995 ref NP_002697.1	protein phosphatase 1B isoform ...	54	5e-06	L
gi 16223994 gb AAL15579.1	hFEM-2 [Homo sapiens]	54	9e-06	L
gi 20978304 gb AAM33411.1	putative protein phosphatase PP2...	53	1e-05	
gi 11282002 pir T47644	protein phosphatase 2C-like protein...	53	1e-05	
gi 546853 gb AAB30830.1	Tpdlp=protein phosphatase 2C homol...	53	2e-05	
gi 18416366 ref NP_568237.1	protein phosphatase 2C (PP2C) ...	52	2e-05	

Alignments

Get selected sequences

Select all

Deselect all

☐ >gi|39573724|ref|NP_945149.1| **L** protein phosphatase 2C eta isoform 2; protein p
[Mus musculus]

gi|37704769|gb|AAR01612.1| **L** protein phosphatase 2C eta [Mus musculus]
Length = 406

Score = 704 bits (1816), Expect = 0.0

Identities = 345/387 (89%), Positives = 366/387 (94%), Gaps = 3/387 (0%)

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+INA EKSEFNEDQAACGKLCIRRCEFG EE EWL T+CPEEF LTGHYWALFDGHGGPAAA

Sbjct: 20 VINA EKSEFNEDQAACGKLCIRRCEFGIEEDQEWLTVCP EEF LTGHYWALFDGHGGPAAA 79

Query: 134 ILAANTLH SCLRRQLEAVVEGLVATQPPMHLN GRCICPSDPQFVEEKGIRAEDLVIGALE 193

ILAANTLH SCLRRQLEAVVEG++A QPPMHL+GRC+CPSDPQFVEEKGI+AEDLVIGALE

Sbjct: 80 ILAANTLH SCLRRQLEAVVEGMMAPQPPMHL SGRVCPSDPQFVEEKGIQAEDLVIGALE 139

Query: 194 SAFQECDEVIGRELEASGQMG GCTALVAVSLQ GKLYMANAGDSRAILVRRDEIRPLSFEF 253

+AFQECD+VIGRELEASGQ+GGCTALVAV LQ GKLY+ANAGDSRAILVRR EIR LS EF

Sbjct: 140 NAFQECDDVIGRELEASGQVGGCTALVAVFLQ GKLYVANAGDSRAILVRRHEIRQLSSEF 199

Query: 254 TPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHHMSGWSYKRVEKS 313
 TPETERQRIQQALAF YPELLAGEFTRLEFPRLKGGDLGQKVLFRDHHM GWSYKRVEKS
 Sbjct: 200 TPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHHMRGWSYKRVEKS 259

Query: 314 DLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKPFLLSVPQVTVLVDVDQLEL 373
 DLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD+IQLKPFLLS+PQVTVLVDV QL +
 Sbjct: 260 DLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTDIQLKPFLLSIPQVTVLVDVHQLAV 319

Query: 374 QEEDVVVVMATDGLWDVLSNEQVAWLVRSLPGNQ-EDPHRFSKLAQMLIHSTQGKEDSLT 432
 QE+DVVVVMATDGLWDVLSNEQVA LVRSL GNQ +DPHRFS+LA+MLIH+TQGK++ T
 Sbjct: 320 QEEDVVVVMATDGLWDVLSNEQVALLVRSLTGNQKDDPHRFSELAQMLIHNTQGKDNGAT 379

Query: 433 EEGQVSYDDVSVFVIPLHSQGESSDH 459
 EEGQVSYDDVSVFVIPLHSQ QE S H
 Sbjct: 380 GEGQVSYDDVSVFVIPLHSQAQEGSGH 406

☐ >gi|26338027|dbj|BAC32699.1| ☒ unnamed protein product [Mus musculus]
 gi|26378394|dbj|BAB28679.2| ☒ unnamed protein product [Mus musculus]
 gi|34849561|gb|AAH58248.1| ☒ RIKEN cDNA 2810423019 [Mus musculus]
 Length = 306

Score = 544 bits (1402), Expect = e-153
 Identities = 269/306 (87%), Positives = 288/306 (94%), Gaps = 1/306 (0%)

Query: 155 LVATQPPMHLNGRCICPSDPQFVEEKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMG 214
 ++A QPPMHL+GRC+CPSPDPQFVEEKGI+AEDLVIGALE+AFQECD+VIGRELEASGQ+G
 Sbjct: 1 MIAPQPPMHLNGRCICPSDPQFVEEKGIQAEDLVIGALENAFQECDDVIGRELEASGQVG 60

Query: 215 GCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPELLA 274
 GCTALVAV LQGKLY+ANAGDSRAILVRR EIR LS EFTPETERQRIQQALAF YPELLA
 Sbjct: 61 GCTALVAVFLQGKLYVANAGDSRAILVRRHEIRQLSSEFTPETERQRIQQALAFVYPELLA 120

Query: 275 GEFTRLEFPRLKGGDLGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLA 334
 GEFTRLEFPRLKGGDLGQKVLFRDHHM GWSYKRVEKSDLKYPLIHGQGRQARLLGTLA
 Sbjct: 121 GEFTRLEFPRLKGGDLGQKVLFRDHHMRGWSYKRVEKSDLKYPLIHGQGRQARLLGTLA 180

Query: 335 VSRGLGDHQLRVLDTNQLKPFLLSVPQVTVLVDVDQLELQEEDVVVVMATDGLWDVLSNEQ 394
 VSRGLGDHQLRVLD+IQLKPFLLS+PQVTVLVDV QL +QE+DVVVVMATDGLWDVLSNEQ
 Sbjct: 181 VSRGLGDHQLRVLDTDIQLKPFLLSIPQVTVLVDVHQLAVQEEDVVVVMATDGLWDVLSNEQ 240

Query: 395 VAWLVRSLPGNQ-EDPHRFSKLAQMLIHSTQGKEDSLTEEGQVSYDDVSVFVIPLHSQG 453
 VA LVRSL GNQ +DPHRFS+LA+MLIH+TQGK++ T EGQVSYDDVSVFVIPLHSQ
 Sbjct: 241 VALLVRSLTGNQKDDPHRFSELAQMLIHNTQGKDNGATGEGQVSYDDVSVFVIPLHSQA 300

Query: 454 QESSDH 459
 QE S H
 Sbjct: 301 QEGSGH 306

☐ >gi|21389471|ref|NP_653242.1| ☒ hypothetical protein FLJ32332 [Homo sapiens]
 gi|16552416|dbj|BAB71302.1| ☒ unnamed protein product [Homo sapiens]
 Length = 270

Score = 512 bits (1318), Expect = e-144

Identities = 251/252 (99%), Positives = 252/252 (100%)

Query: 162 MHLNGRCICPSDPQFVEEKGIRAEDLVIGALESASFQECDEVIGRELEASGQMGGCTALVA 221
 MHLNGRCICPSDPQFVEEKGIRAEDLVIGALESASFQECDEVIGRELEASGQMGGCTALVA
 Sbjct: 1 MHLNGRCICPSDPQFVEEKGIRAEDLVIGALESASFQECDEVIGRELEASGQMGGCTALVA 60

Query: 222 VSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPELLAGEFTRLE 281
 VSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPELLAGEFTRLE
 Sbjct: 61 VSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPELLAGEFTRLE 120

Query: 282 FPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGTLAVSRGLGD 341
 FPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGTLAVSRGLGD
 Sbjct: 121 FPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGTLAVSRGLGD 180

Query: 342 HQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 401
 HQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR
 Sbjct: 181 HQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 240

Query: 402 FLPGNQEDPHRF 413
 FLPGNQEDPHR+
 Sbjct: 241 FLPGNQEDPHRY 252

☐ >gi|16307111|gb|AAH09644.1| ☒ FLJ32332 protein [Homo sapiens]
 Length = 247

Score = 499 bits (1286), Expect = e-140
 Identities = 247/247 (100%), Positives = 247/247 (100%)

Query: 213 MGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPEL 272
 MGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPEL
 Sbjct: 1 MGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPEL 60

Query: 273 LAGEFTRLEFPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGT 332
 LAGEFTRLEFPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGT
 Sbjct: 61 LAGEFTRLEFPRRLKGDDLQKQVLFVDHMHMSGWSYKRVEKSDLYPLIHGQGRQARLLGT 120

Query: 333 LAVSRGLGDHQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSN 392
 LAVSRGLGDHQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSN
 Sbjct: 121 LAVSRGLGDHQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSN 180

Query: 393 EQVAWLVRSLPGNQEDPHRFSKLAQMLIHSTQGKEDSLTEEGQVSYDDVSVFVIPLHSQ 452
 EQVAWLVRSLPGNQEDPHRFSKLAQMLIHSTQGKEDSLTEEGQVSYDDVSVFVIPLHSQ
 Sbjct: 181 EQVAWLVRSLPGNQEDPHRFSKLAQMLIHSTQGKEDSLTEEGQVSYDDVSVFVIPLHSQ 240

Query: 453 GQESSDH 459
 GQESSDH
 Sbjct: 241 GQESSDH 247

☐ >gi|12856386|dbj|BAB30649.1| ☒ unnamed protein product [Mus musculus]
 Length = 254

Score = 445 bits (1145), Expect = e-124
 Identities = 223/254 (87%), Positives = 235/254 (92%), Gaps = 1/254 (0%)

Query: 207 LEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQAL 266

Sbjct: 1 LEA GQ+GGCTALVAV LQ GKLY+ANAGDSRAILVRR EIR LS EFTPETERQRIQQLA
LEALGQVGGCTALVAVFLQ GKLYVANAGDSRAILVRRHEIRQLSSEFTPETERQRIQQLA 60

Query: 267 FVYPELLAGEFTRLEFPRLKGGDDLQ GKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQ 326
F YPELLAGE TRLEFPRLKGGDDLQ GKVLFRDHHM GWSYKRVEKSDLKYPLIHGQGRQ

Sbjct: 61 FTYPELLAGELTRLEFPRLKGGDDLQ GKVLFRDHHMRGWSYKRVEKSDLKYPLIHGQGRQ 120

Query: 327 ARLLGTLAVSRGLGDHQLRVLDTNLQ LKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGL 386
ARLLGTLAVSRGLGDHQLRVLD+IQLKPFLLS+PQVTVLDV QL +QE+DVVVMATDGL

Sbjct: 121 ARLLGTLAVSRGLGDHQLRVLDTDIQLKPFLLSIPQVTVLDVHQLAVQEEDVVVMATDGL 180

Query: 387 WDVLSNEQVAWLVRSLPGNQ-EDPHRF SKLAQMLIHSTQ GKEDSLTEEGQVSYDDVSVF 445
WDVLSNEQVA LVRSL GNQ +DPHRFS+LA+MLIH+TQ GK++ T EGQVSYDDVSVF

Sbjct: 181 WDVLSNEQVALLVRSLTGNQKDDPHRFSELAKMLIHNTQ GKDNATGEGQVSYDDVSVF 240

Query: 446 VIPLHSQGESSDH 459
VIPLHSQ QE S H

Sbjct: 241 VIPLHSQAQEGSGH 254

☐ >gi|28892713|ref|NP_795893.1| ☒ RIKEN cDNA A430075L18 gene [Mus musculus]
☒ gi|26333637|dbj|BAC30536.1| ☒ unnamed protein product [Mus musculus]
Length = 469

Score = 357 bits (917), Expect = 2e-97

Identities = 206/433 (47%), Positives = 266/433 (61%), Gaps = 49/433 (11%)

Query: 30 PYRRPFLRGSSSSPGAADASRRPDSRPVRSARGRTL PWNAGYAEIINA EKSEFNEDQA 89
PY RP FL S + S +RP+ R LPW GYAE+INA KS NEDQA

Sbjct: 40 PYGRPEFL---GLSQDEVECSADHIA RPILILKETRRLPWATGYAEVINAGKSTHNEDQA 96

Query: 90 ACGKLCIR-----RCEFGAEEEWLTL---CPEEFLTGHYWALFDGHGGPA 131
+C L ++ R E L L E ++ HYW+LFDGH G

Sbjct: 97 SCEVLTVKKKAGTITSTPNRNSKRSSLPNGEGLQLKENSESEGISCHYWSLFDGHAGSG 156

Query: 132 AAILAANTLHSLRRQLEAVVEGL--VATQPPMHL-----NGRCIC----- 170
AA++A+ L + +QL+ +VE L A PP L +GR +

Sbjct: 157 AAVVASRLQLQHHTQQQLQDIVEILKNSAILPPTCLGEEPESTPAHGRTLTRAASLRGGVG 216

Query: 171 ----PSDP--QFVEEKGIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVSL 224
PS P +F EK I E LVIGALES AF+E D I RE A GGCTAL+ V L

Sbjct: 217 APGSPSTPPTRFFTEKKIPHECLVIGALES AFKEMDLQIERERSAYNISGGCTALIVVCL 276

Query: 225 QGKLYMANAGDSRAILVRRDEIRPLSF EFTPETERQRIQQLAFVYPELLAGEFTRLEFP 284
GKLY+ANAGDSRAI++R EI P+S EFTPETERQR+Q LAF+ P LL EFT LEFP

Sbjct: 277 LGKLYVANAGDSRAIIRNGEII PMSSEFTPETERQLQLAFMQPHLLGNEFTHLEFP 336

Query: 285 RLKGGDDLQ GKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQL 344
R++ +LG+K+L+RD +M+GW+YK +E DLK+PLI+G+G++AR++ T+ V+RGLGDH L

Sbjct: 337 RVQRKELGKKMLYRDFNMTGWAYKTIEDDDLKFPLIYGEGKKARVMATIGVTRGLGDHDL 396

Query: 345 RVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLP 404
+V D+NI +KPFL S P+V V D+ + E DDV+++ATDGLWDVLSNE+VA + FL P

Sbjct: 397 KVHDSNIYIKPFLSSAPEVRVYDLSRYEHGADDVLILATDGLWDVLSNEEVAEAITQFLP 456

Query: 405 G-NQEDPHRF SKL 416
+ +DPHRF L

Sbjct: 457 NCDPDDPHRFVPL 469

☐ >gi|29611652|ref|NP_082258.1| ☒ protein phosphatase 2a, catalytic subunit, zeta
musculus]

gi|29373411|gb|AA072079.1| ☒ protein phosphatase 2C zeta [Mus musculus]
Length = 507

Score = 335 bits (858), Expect = 1e-90

Identities = 203/469 (43%), Positives = 274/469 (58%), Gaps = 33/469 (7%)

Query: 12 PGEPLPAPRPPGPHASPVYRRPRFLRGSSSSPGAADASRRPD--SRPVRSPA-RGRTL 68
PG + AP+ + V + RP FL+ SPG R D R V+SP GR LP
Sbjct: 49 PGNQVGAPQ----KTATVSFSRPTFLQ---LSPGGETLRRADDHAGRAVQSPPDTRRLP 101

Query: 69 WNAGYAEIINA EKSEFNEDQAACGKLCIR---RCEFGAEEEWLTLCPEEFLTGHYWALF 124
W+ GYAE+INA KS NEDQA C + + R G E + +YW LF
Sbjct: 102 WSTGYAEVINAGKSRHNEDQACCEVVYVESRRRSVTVGSRE---PSHNQGFYFWGLF 158

Query: 125 DGHGGPAAAILAANTLHSLRRQLEAVVEGL----VATQPPMHLNGRCICPSDPQFVE-- 178
DGH G AA +A+ LH +R QL+ +VE L G PS Q V
Sbjct: 159 DGHAGGGAAEMASRLLRHIREQLKDLVEILKDPLPPPLCLPSTPGTPGAPSPSQLVSPQ 218

Query: 179 -----EKGIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANA 233
+K + + L++GA+E+AF DE + RE GGC ALV + L GK+Y+ANA
Sbjct: 219 SCWSPQKEVTHDSLIVGAIENAFHLMDEQMARERRGHQVEGGCCALVVLYLLGKMYVANA 278

Query: 234 GDSRAILVRRDEIRPLSFEFTPETERQRIQQALAFVYPELLAGEFTTRLEFPRLKGGDDLQ 293
GDSRAI+VR EI P+S EFTPETERQR+Q L F+ PELL EFT LEFPRR++ +LGQ
Sbjct: 279 GDSRAIIVRNGEIIIPMSREFTPETERQRLQLLGFLKPELLGSEFTHLEFPRRVQPKELGQ 338

Query: 294 KVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNLQ 353
++L+RD +M+GW+YK++E DL++PL+ G+G++AR++ T+ V+RGLGDH L+V + + +
Sbjct: 339 RMLYRDQNMGTWAYKKIEVEDLRFPLVCGEGKKARVMATIGVTRGLGDHNLKVCSSSTLSI 398

Query: 354 KPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPQNG-EDPHR 412
KPFL P+V V D+ Q E DDV+V+ TDGLWDV ++ +VA V L + DP R
Sbjct: 399 KPFLSCFPPEVRVYDLTQYEHCPDDVLVLGTDGLWDVTNDSEVAATVDRVLSSYEPNDPSR 458

Query: 413 FSKLAQMLIHSTQG----KEDSLTEEGQVSYDDVSFVFIPLHSQGOESS 457
++ LAQ L+ +G + L S DD+SVFVIPL G S
Sbjct: 459 YTALAQALVLGARGIPDRDGRWLPNNKLGSGDDISVFVIPLGGPGSSYS 507

☐ >gi|33873319|gb|AAH11803.2| ☒ MGC19531 protein [Homo sapiens]
Length = 488

Score = 334 bits (857), Expect = 2e-90

Identities = 202/452 (44%), Positives = 269/452 (59%), Gaps = 39/452 (8%)

Query: 31 YRRPRFLRGSSSSPGAADASRRPDSPVRSPA-RGRTL PWNAGYAEIINA EKSEFNEDQA 89
+ RP FL+ SPG + R V+SP GR LPW+ GYAE+INA KS NEDQA
Sbjct: 50 FSRPTFLQ---LSPGGLRRADDHAGRAVQSPPDTRRLPWSTGYAEVINAGKSRHNEDQA 106

Query: 90 ACGKLCI--RRCEFGAEEEWLTLCPE--EFLTGHYWALFDGHGGPAAAILAANTLHSLR 145
C + + RR G E P + L +YW LFDGH G AA +A+ LH +R
Sbjct: 107 CCEVVYVEGRRSVTVGPPE-----PSRGQGLCFYFWGLFDGHAGGGAAEMASRLLRHIR 161

Query: 146 RQLEAVVE-----GLVATQPPMHLNGRCICPSDPQFVEEKGIRAEDLVI 189
 QL+ +VE G + P HL G C S +K + E LV+

Sbjct: 162 EQLKDLVEILQDPSPPPLCLPTTPGTPDSSDSPSHLLGPQSCWS-----SQKEVSHESLVV 216

Query: 190 GALESAFQECDEVIGRELEASQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPL 249
 GA+E+AFQ DE + RE GGC ALV + L GK+Y+ANAGDSRAI+VR EI P+

Sbjct: 217 GAENAFQLMDEQMARERRGHQVEGGCCALVVIYLLGKVYVANAGDSRAIIVRNGEIIIPM 276

Query: 250 SFEFTPETERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDLGQKVLFRDHHMSGWSYKR 309
 S EFTPETERQR+Q L F+ PELL EFT LEFPRR+ +LGQ++L+RD +M+GW+YK+

Sbjct: 277 SREFTPETERQRLQLLGLFKPELLGSEFTHLEFPRRVLPKELGQRMLYRDQNMGTWAYKK 336

Query: 310 VEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDNIQLKPFLLSVPQVTVLDVD 369
 +E DL++PL+ G+G++AR++ T+ V+RGLGDH L+V + + +KPFL P+V V D+

Sbjct: 337 IELEDLRFPLVCGEGKKARVMATIGVTRGLGDHSLKVCSSTLPIKPFLLSCFPEVRVYDLT 396

Query: 370 QLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPH-RFSKLAQMLIHSTQGKE 428
 Q E DDV+V+ TDGLWDV ++ +VA V L + + H R++ LAQ L+ +G

Sbjct: 397 QYEHCPDDVLVLGTDGLWDVTTDCEVAATVDRVLSAYEPNDHSRYTALAQAALVLGARGTP 456

Query: 429 DS-----LTEEGQVSYDDVSVFVIPLHSQGQES 456
 L S DD+SVFVIPL G S

Sbjct: 457 RDRGWRLPNKLGSGDDISVFVIPLGGPGSYS 488

>gi|31127152|gb|AAH52910.1| Unknown (protein for IMAGE:30075932) [Mus musculus]
 Length = 424

Score = 332 bits (850), Expect = 1e-89

Identities = 182/368 (49%), Positives = 245/368 (66%), Gaps = 32/368 (8%)

Query: 114 EFLTGHYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGL--VATQPPMHL----- 164
 E ++ HYW+LFDGH G AA++A+ L + +QL+ +VE L A PP L

Sbjct: 50 EGISCHYWSLFDGHAGSGAAVVASRLQHPIITQQLQDIVEILKNSAILPPTCLGEEPEST 109

Query: 165 --NGRCIC-----PSDP--QFVEEKGIRAEDLVIGALESAFQECDEVIGRE 206
 +GR + PS P +F EK I E LVIGALESAF+E D I RE

Sbjct: 110 PAHGRTLTRAASLRGGVGAPGSPSTPPTRFFTEKKIPHECLVIGALESAFKEMDLQIERE 169

Query: 207 LEASQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQLA 266
 A GGCTAL+ V L GKLY+ANAGDSRAI++R EI P+S EFTPETERQR+Q LA

Sbjct: 170 RSAYNISGGCTALIVVCLLGKLYVANAGDSRAIIRNGEIIIPMSSEFTPETERQRLQYLA 229

Query: 267 FVYPELLAGEFTRLEFPRLKGDDLGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQ 326
 F+ P LL EFT LEFPRR++ +LG+K+L+RD +M+GW+YK +E DLK+PLI+G+G++

Sbjct: 230 FMQPHLLGNEFTHLEFPRRVQRKELGKKMLYRDFNMTGWAYKTIEDDDLKFPLIYGEGKK 289

Query: 327 ARLLGTLAVSRGLGDHQLRVLDNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGL 386
 AR++ T+ V+RGLGDH L+V D+NI +KPFL S P+V V D+ + E DDV+++ATDGL

Sbjct: 290 ARVMATIGVTRGLGDHDLKVHDSNIYIKPFLLSAPEVRVYDLSTRYEHGADDVLILATDGL 349

Query: 387 WDVLSNEQVAWLVRSLPG-NQEDPHRFSKLAQMLIHSTQG----KEDSLTEEGQVSYDD 441
 WDVLSNE+VA + FL + +DPHR++ AQ L+ +G + ++ + S DD

Sbjct: 350 WDVLSNEEVAEAITQFLNCDPDDPHRYTLAAQDLVMRARGVLKDRGWRISNDRLGSGDD 409

Query: 442 VSVFVIPL 449
 +SV+VIPL

Sbjct: 410 ISVYVIPL 417

☐ >gi|34859725|ref|XP_215663.2| ☒ similar to protein phosphatase 2C zeta [Rattus r
Length = 530

Score = 327 bits (838), Expect = 3e-88

Identities = 203/491 (41%), Positives = 278/491 (56%), Gaps = 56/491 (11%)

Query: 13 GEPLPAPRPPGPHASVPYRRPRFLRGSSSSPGAADASRRPDSRPVRSRPA-RGRTLFPWNA 71
G +PAP+ +PV + RP FL+ SPG + R V+SP GR LPW+
Sbjct: 50 GNEIPAPQKTA--ETPVSFSRPTFLQ---LSPGGLRRADDHVGRAVQSPPDGTGRRLPWST 104

Query: 72 GYAEIINA EKSEFNEDQAACGKLCI--RRCEFGAEEEWLTLCP EEF LTGHY WALFDGHGG 129
GYAE+INA KS NEDQA C + + RR G E + + +YW LFDGH G
Sbjct: 105 GYAEVINAGKSRHNEDQACCEVVYVESRRSITGVSR---PSHNQGF SFYYWGLFDGHAG 161

Query: 130 PAAAILAANTLH SCLRRQLEAVVEGL-----VATQPPMHLN GRCICPSDPQFV 177
AA +A+ LH +R QL+ +VE L P + + + P +
Sbjct: 162 GGAAEMASRL LHRHIREQLKDLVEILQDPLPPPLCLPSTPGTPGVSSPSQLVSPQ--SWS 219

Query: 178 EEKGIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDS- 236
+K + + LV+GA+E+AFQ DE + RE GGC ALV V L GK+Y+ANAGDS
Sbjct: 220 PQKEVTHDSL VVGAIENAFQLMDEQMARERRGHLVEGGCCALVVVYLLGKMYVANAGDSS 279

Query: 237 -----RAILVRRDEIRPLSFEFTPETERQRIQQLAFVYPE 271
RAI+VR EI P+S EFTPETERQR+Q L F+ PE
Sbjct: 280 PPAWNSPLCMNFSEKARSKSFLSLGRAIIVRNGEIIIPMSREFTPETERQRLQLLGFLKPE 339

Query: 272 LLAGEFTRLEFP RRLKGDDLQKQVLF RDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLG 331
LL EFT LEFPRR++ +LGQ++L+RD +M+GW+YK++E DL++PL+ G+G++AR++
Sbjct: 340 LLGSEFTHLEFP RRVQPKELGQRMLYRDQNM TGWAYKKIELEDLRFP LVC GEGKKARVMA 399

Query: 332 TLAVSRGLGDHQLRVLD TNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLS 391
T+ V+RGLGDH L+V + + +KPFL P+V V D+ Q E DDV+V+ TDGLWDV +
Sbjct: 400 TIGVTRGLGDHNLKVCSS TLPKPFLLSCFP EVRVYDLTQYEHCPDDVLVLGTDGLWDVTN 459

Query: 392 NEQVAWLVR SFLPGNQ-EDPHRF SKLAQM LIHSTQG----KEDSLTEEGQVS YDDVS VFV 446
+ +VA V L + DP R++ LAQ L+ +G + L S DD+SVFV
Sbjct: 460 DSEVAATVDRVLSTYEPNDPSRYTALAQA LVLGARGIPRDRGWRLPNNKLGSGDDISVFV 519

Query: 447 IPLHSQGQESS 457
IPL G S
Sbjct: 520 IPLGGPGSSYS 530

☐ >gi|34526234|dbj|BAC85206.1| unnamed protein product [Homo sapiens]
Length = 169

Score = 311 bits (796), Expect = 2e-83

Identities = 155/156 (99%), Positives = 155/156 (99%)

Query: 304 GWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TNIQLKPFLLSVPQV 363
G SYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TNIQLKPFLLSVPQV
Sbjct: 14 GRSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TNIQLKPFLLSVPQV 73

Query: 364 TVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR SFLPGNQEDPHRF SKLAQM LIHS 423
TVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR SFLPGNQEDPHRF SKLAQM LIHS
Sbjct: 74 TVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR SFLPGNQEDPHRF SKLAQM LIHS 133

Query: 424 TQ GKEDSLTEEGQVS YDDVSVFVIPLHSQGGQESSDH 459
TQ GKEDSLTEEGQVS YDDVSVFVIPLHSQGGQESSDH
Sbjct: 134 TQ GKEDSLTEEGQVS YDDVSVFVIPLHSQGGQESSDH 169

☐ >gi|39930533|ref|NP_005158.4| ☒ L hypothetical protein MGC19531 [Homo sapiens]
gi|11360166|pir||T43442 hypothetical protein DKFZp434P1514.1 - human
gi|6599183|emb|CAB63764.1| ☒ L hypothetical protein [Homo sapiens]
Length = 299

Score = 271 bits (692), Expect = 3e-71
Identities = 141/287 (49%), Positives = 192/287 (66%), Gaps = 5/287 (1%)

Query: 175 QFVEEKGIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAG 234
Q + K + E LV+GA+E+AFQ DE + RE GGC ALV + L GK+Y+ANAG
Sbjct: 13 QLKDLKEVSHESLVVGAIENAFQLMDEQMARERRGHQVEGGCCALVVIYLLGKVVANAG 72

Query: 235 DSRAILVRRDEIRPLSF EFTPETERQRIQQ LAFVYPELLAGEFTRLEFP RRLKGDDLQK 294
DSRAI+VR EI P+S EFTPETERQR+Q L F+ PELL EFT LEFPRR+ +LGQ+
Sbjct: 73 DSRAIIVRNGEIIPMSREFTPETERQRLQLLGLFLKPELLGSEFTHLEFP RRVLPKELGQR 132

Query: 295 VLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TN IQLK 354
+L+RD +M+GW+YK++E DL++PL+ G+G++AR++ T+ V+RGLGDH L+V + + +K
Sbjct: 133 MLYRDQNMTGWAYKKIELEDLRFPLVC GEGKKARVMATIGVTRGLGDHSLKVCSS TLPIK 192

Query: 355 PFLLSVPQVTVLVDVQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLP GNPQEDPH-RF 413
PFL P+V V D+ Q E DDV+V+ TDGLWDV ++ +VA V L + + H R+
Sbjct: 193 PFLSCFPEVRVYDLTQYEHCPDDVLVLGTDGLWDVTTDCEVAATVDRVLSAYEPNDHSRY 252

Query: 414 SKLAQMLIHSTQ GKEDS----LTEEGQVS YDDVSVFVIPLHSQGGQES 456
+ LAQ L+ +G L S DD+SVFVIPL G S
Sbjct: 253 TALAQALVLGARGTPRDRGWRLPNNKLGSGDDISVFVIPLGGPGSYS 299

☐ >gi|17539110|ref|NP_502329.1| ☒ L protein phosphatase 2C-like (4N30) [Caenorhabditis
gi|7497315|pir||T32903 hypothetical protein C42C1.2 - Caenorhabditis elegans
gi|2804429|gb|AAB97544.1| Hypothetical protein C42C1.2 [Caenorhabditis elegans]
Length = 582

Score = 261 bits (666), Expect = 2e-68
Identities = 178/509 (34%), Positives = 261/509 (51%), Gaps = 93/509 (18%)

Query: 30 PYRRPRFLRGSSSSPG-AADASRRPDSRPVRS PARGRTL PWNAGYAEI INAEKSEFNEDQ 88
PY RP FL S ++D S RP P R P R +P GYAE INA K+ NEDQ
Sbjct: 56 PYSRPEFLYFSEEEIALSSDHSVRPVLCP-RFPHR---MPLYVG YAEAINAGKTVQ NEDQ 111

Query: 89 AACGKLCIRRCE-----FGAE E-----WLTLCPEEF 115
A+ L + + + F ++E+ +L + E+
Sbjct: 112 ASAKMLVLTQH QGNEMNGFSSDEKKSETRKRNSNENDDDPMLTPGGDDTVSYLNVVNEKS 171

Query: 116 -----LTGHYWALFDGHG GPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLN----- 165
G ++LFDGH G A A++A+ LH ++ +L V++ ++ +LN
Sbjct: 172 SMFAPRADGALFSLFDGHAGSAVAVVASKCLHEHVKSRLCEVLD TILHMDRHNENLNF GK H 231

Query: 166 --GRCICPSDPQFVEEKGIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVS 223
S+ +E IR+E LV GALE+AF + DE I ++ + GGC + +

Sbjct: 232 RSESSYSMSEMSREDENRIRSEHLVKGALETAFLDMDEQIAQDKQVWRLPGGCAVISVLV 291

Query: 224 LQGKLY-----MANAGDSRAILVRRDEIRPL 249
 GKLY ++NAGD RAILV D R L

Sbjct: 292 FLGKLYIGGDFKNFFELSEGCHITIFPNSINVEKINNTRKLSNAGDCRAILVTS DGSRAL 351

Query: 250 SFEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHHMSGWSYKR 309
 S + TP +ER+R+Q+LA+ PEL+ F+RLE+ +RL DL +VL+RD M GW+ K

Sbjct: 352 SKDLTPASERKRLQELAYRNPELIGNSFSRLEYSKRLTIHDLKSRVLYRDWFMDGWAVKT 411

Query: 310 VEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKPFLLSVPQVTVLDV- 368
 V++ DL+ LI R+ RLL T+ VSRG GDH L +D + +KPFL +VP+++V ++

Sbjct: 412 VKECDLRPSLISETSRKKRLNLTIGVSRGFGDHLLTVDERLSIKPFLSAVPEISVTNLR 471

Query: 369 DQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSL- PGNQEDPHRFSKLAQMLIHSTQGK 427
 D L + DVV++A+DGLWDVLSNE +VRS L + DP R+++ AQ L+ + +G+

Sbjct: 472 DMNTLTDKDVVIVASDGLWDVLSNEDAGLIVRSTLGSTDSADPSRYTQAAQDLVAAARGQ 531

Query: 428 EDS-----LTEEGQVSYDDVSVFVIPL 449
 + S + G SYDD++VFVIPL

Sbjct: 532 QASGNLKRWMNTGGHASYDDITVFVIPL 560

☐ >gi|31203861|ref|XP_310879.1| ENSANGP00000008051 [Anopheles gambiae]
 gi|30177994|gb|EAA06421.2| ENSANGP00000008051 [Anopheles gambiae str. PEST]
 Length = 429

Score = 256 bits (654), Expect = 6e-67

Identities = 161/439 (36%), Positives = 243/439 (55%), Gaps = 36/439 (8%)

Query: 31 YRRPRFLRGSSSSPGAADASRRPDSRPVRSPARGRTL PWNAGYAEIINA EKSEFNEDQAA 90
 Y RP FL+ + A A + RP+ P LPW+ GYAE +N+ KS++NEDQAA

Sbjct: 6 YARPPFLQLLT YDELKASADH--NVRPIIVPRDISLLPWSTGYAECVNSGKSKWNEDQAA 63

Query: 91 CGKLCIRRCFEFGAEEEWLTLCP EEFLTGHY WALFDGHGGPAAAILAANTLHSLRRQLEA 150
 + + + L Y+ ++DGH G AA+ AAN H L +L

Sbjct: 64 FHRQVLSHPSRQYHD-----LPYTYFGIYDGHAGYGAALAAANQFHYILHEKLVD 113

Query: 151 VVEGLVATQPPMHLNG-----RCICPSDPQFVEEKGIRAEDLVIGALES AFQECDEVIGR 205
 V++ L+ P + +G P F K + ++L++GALE+AF + D V+

Sbjct: 114 VIDLLM---PRVEGDGGGLALHATLPHPSLF--HKQVSKDELIVGALEAAAFADMDAVLAE 168

Query: 206 ELEASQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEFTPETERQRIQQ 265
 + + GGCTALVA+ + GKL++ANAGDSR +L +R P SF+ TP+TER R+ +

Sbjct: 169 DRDKYRNAGGCTALVALFILGKLFVANAGDSRGVLCKRVVAEPCSF DHTPDTERSRLTV 228

Query: 266 AFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGR 325
 P LL GE+ +E+ ++ DLG ++L+R M GW+YK + +DLK PLI G G+

Sbjct: 229 GKHNPA LLGGEYIAMEYAKKPTTKDLGARILYRQGAMKGWYTKTLTATDLKIPLITGVGK 288

Query: 326 QARLLGTLAVSRGLGDHQLRVLDTNQLKPFLLSVPQVTVLDVDQLELQEDD----- 377
 ++RLLGT+ V+RG GDH L+ L +N+ +KPFL + P V D+ Q+ + D

Sbjct: 289 RSRL LGTIGVTRGFGDHD LKALGSNLPIKPFLSAHPDVVCFDLAQVRSEPADENS DGEYG 348

Query: 378 VVVMATDGLWDVLSNEQVAWLVRSL PGNQEDPHRFSKLAQMLIHSTQ GKEDS-----L 431
 ++VMATDGLWDV ++QVA V L + HR++ +AQ L+ ++G+ +

Sbjct: 349 ILVMATDGLWDVSESQQVANTVFGLTKRFPAERHRYTMVAQELVARSRGRANESGHWRLS 408

Query: 432 TEEGQVSYDDVSVFVIPLH 450

+ DD+SV VIP+H

Sbjct: 409 DSRAAATVDDISVIVIPVH 427

☐ >gi|19920426|ref|NP_608468.1| ☒ CG17598-PA [Drosophila melanogaster]
 gi|17944215|gb|AAL48002.1| ☒ GM14138p [Drosophila melanogaster]
 gi|22832704|gb|AAF50944.2| ☒ CG17598-PA [Drosophila melanogaster]
 Length = 651

Score = 255 bits (651), Expect = 1e-66

Identities = 165/467 (35%), Positives = 247/467 (52%), Gaps = 53/467 (11%)

Query: 24 PHASVPYRRPRFLRGSSSSPGAADASRRPDSRPVRSFARGRTLFPWNAGYAEIINA EKSE 83
 P PY RP FL+ +P AS + RP+ P LPW GYAE +N+ KSE
 Sbjct: 52 PMPDKFPYARPPFLQ--LLTPDELASADHNVRPIIVPRDINLLPWGTGYAECVNSGKSE 109

Query: 84 FNEDQAACGKLCIRRCEFGAEEEWLTLCPPEEFLTGHYWALFDGHGGPAAAILAANTLHSC 143
 +NEDQ A + + E + L Y+ +FDGH G AA+ A++ H
 Sbjct: 110 WNEDQGAFCRQVLSDEHKHPD-----LPYTYFGIFDGHAGYGAALAASHQFHFI 159

Query: 144 LRRQLEAVVEGLV---ATQPPMHLNGRCICPSDPQFVE-EKGIRAEDLVIGALESASFQEC 199
 L +L +E L+ A G + P+ P + ++ + ++L+IGALESASF
 Sbjct: 160 LHEKLVDCLLELLPRDADATNGGGEGNKLNPFTFPHPIYFQRRVTKDELIIGALESAFFNM 219

Query: 200 DEVIGRELEASQMGGCTALVAVSLQGKLYMANAGDSRAILVRR----- 243
 D +I ++ + GGCTA V++ + GK+Y+ANAGDSRA+L +R
 Sbjct: 220 DSLIAQDCDRYRDAGGCTACVSLFIDGKMYVANAGDSRAVLCQRRATPERPQTNTDSGIE 279

Query: 244 -DEIR-----PLSFEEFTPETERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDLQKQVL 296
 D + P S + TPETER+R+ +A + P L+ + +E+ +R D+GQ++L
 Sbjct: 280 PDPLEASCYPVPFSAHPTETERERLLNVARLKPMLMGKHYVAMEYAKRPHIKDMGQRIL 339

Query: 297 FRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDNTNIQLKPF 356
 R M GW+YK + DL P+++G+G+++RLLGTL V+RG GDH+L ++T IQ+KPF
 Sbjct: 340 CRQGTMRGWYKTLTMDLCPVNVNNEGKRSRLGLVTRGFGDHELLAINTGIQIKPF 399

Query: 357 LLSVPQVTVDVDQLELQEDD-----VVMATDGLWDVLSNEQVAWLVRSLPQNQE 408
 L P V D+ Q+ D+ V+VMATDGLWDV N+ V+ V L
 Sbjct: 400 LTPHPDVRQRDLTQVVSIPDEDNRDGDYGVLMATDGLWDVSENDVSRVTFQTLISKYST 459

Query: 409 DPHRFSLAQMLIHSTQGK-EDS-----LTEEGQVSYDDVSVFVIPL 449
 + HR++ +AQ L+ +GK DS + + DD+SV VIP+
 Sbjct: 460 EKHRYTMVAQELVARARGKINDSGHWRLADSKAAATVDDISVIVIPV 506

☐ >gi|3242077|emb|CAA05875.1| protein phosphatase 2C [Arabidopsis thaliana]
 Length = 511

Score = 89.4 bits (220), Expect = 1e-16

Identities = 88/352 (25%), Positives = 153/352 (43%), Gaps = 96/352 (27%)

Query: 116 LTGHYWALFDGHGGPAAAILAANTLHSC LRRQLEAVVEGLVATQPPMHLNGRCICPSDPQ 175
 LTGH++ ++DGHGG A + LH L ++E + + L C +
 Sbjct: 234 LTGHFFGVYDGHGGHKVADYCRDRLHFALEEIERIKDEL-----CKRNTG 279

Query: 176 FVEEKGIRAEDLVIGALESASFQECDEVIGRE-----LEA-SGQMGGCTALVAVSLQG 226
 E + ++ + + + E + IGR LEA + + G TA+VA+

Sbjct: 280 --EGRQVQWDKVF TSCFLTVDGEIEGKIGRAVVGSSDKVLEAVASETVGSTAVVALVCSS 337

Query: 227 KLYMANAGDSRAILVRRDEIRPLSF EFTPETERQRIQQ LAFVYPELLAGEFTRLEFP RRL 286
 + ++N GDSRA+L R E PLS + P+ E E+ R+E

Sbjct: 338 HIVVSNCGDSRAVLFRGKEAMPLSVDHKPDRE-----DEYARIE----- 376

Query: 287 KGDDLQKQVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRV 346
 + G KV+ Q + AR+ G LA+SR +GD L

Sbjct: 377 ---NAGGKVI-----QWQGARVFGVLAMSR SIGDRYL-- 405

Query: 347 LDTNIQLKPFLLSVPQVTVLVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR--FLP 404
 KP+++ P+VT + +ED+ +++A+DGLWDV++N++V + R +

Sbjct: 406 -----KPYVIEPEVTFMP----RSREDECLILASDGLWDVMNNQEVCEIARRRILMW 454

Query: 405 GNQEDPHRFSKLAQMLIHSTQGKEDSLT----EEGQVSYDDVSFVIPLHSQ 452
 + ++ + + + Q D L+ ++G S D++S+ VI L +Q

Sbjct: 455 HKKNGAPPLAERGKGIDPACQAAADYLSMLALQKG--SKDNISIIVIDLKAQ 504

☐ >gi|7768151|emb|CAB90633.1| protein phpsphatase 2C (PP2C) [Fagus sylvatica]
 Length = 413

Score = 88.2 bits (217), Expect = 3e-16

Identities = 94/373 (25%), Positives = 154/373 (41%), Gaps = 96/373 (25%)

Query: 94 LCIRRCEFGAEEEWLTLCPEEFLTGHYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVE 153
 +C RR E E+ +++ P H++ +FDGHG A+ + LH ++ ++E+V E

Sbjct: 126 VCGRRREM---EDAVSVHPSVSNNFHFFGVFDGHGCSHVAMRCRDLHLDIVKEEVESVTE 182

Query: 154 GLVATQPPMHLNRCICPSDPQFVEEK-GIRAEDLVIGALES AFQECDEVIGRELEASGQ 212
 G+ R D + E + I+ + + +CD V

Sbjct: 183 GMEWKDTMEKSFD RM----DKEVQEWVRPIKTTNC---RCDVQTPQCD AV----- 225

Query: 213 MGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSF EFTPETERQRIQQ LAFVYPEL 272
 G TA+VA+ K+ ++N GDSRA+L R PLS + P+ P+

Sbjct: 226 --GSTAVVAIVTPEKIIVSNCGDSRAVL CRNGVAFPLSSDHKPD R-----PD- 270

Query: 273 LAGEFTRLEFP RRLKGDDLQKQVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGT 332
 E R++ D G +V++ D AR+LG

Sbjct: 271 ---ELVRIQ-----DAGGRVIYWD-----GARVLGV 293

Query: 333 LAVSRGLGDHQLRVLD TNIQLKPFLLSVPQVTVLVDVDQLELQEDDVVVMATDGLWDVLSN 392
 LA+SR +GD+ L KP+++S P+VT+ D ED+ +++A+DGLWDV+SN

Sbjct: 294 LAMSRAIGDNYL-----KPYVISEPEVTITD----RTAEDECLILASDGLWDVVS N 340

Query: 393 EQVAWLVR SFLPGNQEDPHRFSKLAQMLIHSTQGKEDSLTEEGQV-----SYDDV 442
 E + R L + S M + + D + + S D+V

Sbjct: 341 ETACGVARMCLRAQKPSSPPRSPGNDMAVGAASESSDKACSDASILLTKLALARHSTDNV 400

Query: 443 SVFVIPLHSQGE 455
 SV V+ L Q+

Sbjct: 401 SVVVVDLRRNHQQ 413

☐ >gi|30685388|ref|NP_173199.2| protein phosphatase 2C (PP2C) -related [Arabidops:
 Length = 511

Score = 88.2 bits (217), Expect = 3e-16

Identities = 80/288 (27%), Positives = 131/288 (45%), Gaps = 73/288 (25%)

Query: 115 FLTGHYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNRCICPSDP 174
 +LT H++ ++DGHGG A + +HS L ++E + E L GR + +
 Sbjct: 232 YLTSHFFGVYDGHGGAQVADYCHDRIHSALAEIEIERIKEELCRRNTG---EGRQV-QWEK 287

Query: 175 QFVEEKGIRAEDLVIGAL-ESAFQECDEVIGRELEA-SGQMGGCTALVAVSLQGKLYMAN 232
 FV+ ++ +D V G + D ++ LEA S + G TA+VA+ + ++N
 Sbjct: 288 VFVDCY-LKVDDEVKKGKINRPVVGSSDRMV---LEAVSPETVGSTAVVALVCSSHIIVSN 343

Query: 233 AGDSRAILVRRDEIRPLSFEEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDL 292
 GDSRA+L+R + PLS + P+ E +
 Sbjct: 344 CGDSRAVLLRGKDSMPLSVDHKPDREDE----- 371

Query: 293 QKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTN 352
 Y R+EK+ K +I QG AR+ G LA+SR +GD
 Sbjct: 372 -----YARIEKAGGK--VIQWQG--ARVSGVLAMSRSIGDQ-----Y 404

Query: 353 LKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
 L+PF++ P+VT + +ED+ +++A+DGLWDV+SN++ R
 Sbjct: 405 LEFPVIPDPEVTFMP----RAREDECLILASDGLWDVMSNQEACDFAR 448

☐ >gi|26452332|dbj|BAC43252.1| unknown protein [Arabidopsis thaliana]
 Length = 511

Score = 88.2 bits (217), Expect = 3e-16

Identities = 80/288 (27%), Positives = 131/288 (45%), Gaps = 73/288 (25%)

Query: 115 FLTGHYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNRCICPSDP 174
 +LT H++ ++DGHGG A + +HS L ++E + E L GR + +
 Sbjct: 232 YLTSHFFGVYDGHGGAQVADYCHDRIHSALAEIEIERIKEELCRRNTG---EGRQV-QWEK 287

Query: 175 QFVEEKGIRAEDLVIGAL-ESAFQECDEVIGRELEA-SGQMGGCTALVAVSLQGKLYMAN 232
 FV+ ++ +D V G + D ++ LEA S + G TA+VA+ + ++N
 Sbjct: 288 VFVDCY-LKVDDEVKKGKINRPVVGSSDRMV---LEAVSPETVGSTAVVALVCSSHIIVSN 343

Query: 233 AGDSRAILVRRDEIRPLSFEEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDL 292
 GDSRA+L+R + PLS + P+ E +
 Sbjct: 344 CGDSRAVLLRGKDSMPLSVDHKPDREDE----- 371

Query: 293 QKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTN 352
 Y R+EK+ K +I QG AR+ G LA+SR +GD
 Sbjct: 372 -----YARIEKAGGK--VIQWQG--ARVSGVLAMSRSIGDQ-----Y 404

Query: 353 LKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
 L+PF++ P+VT + +ED+ +++A+DGLWDV+SN++ R
 Sbjct: 405 LEFPVIPDPEVTFMP----RAREDECLILASDGLWDVMSNQEACDFAR 448

☐ >gi|33309516|gb|AAQ03211.1| protein phosphatase 2C [Prunus avium]
 Length = 426

Score = 85.9 bits (211), Expect = 2e-15

Identities = 84/313 (26%), Positives = 134/313 (42%), Gaps = 81/313 (25%)

Query: 118 GHYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFV 177
 H++ +FDGHG A+ + LH ++++LE G + + M R D + V
 Sbjct: 165 AHFYGVFDGHGCSHVALKCKDRLHEIVKQELETE---RSFAKMDDE-V 219

Query: 178 EEKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSR 237
 +E + A+ E +CD V G TA+VAV K+ ++N GDSR
 Sbjct: 220 QEGNLVAQGPNC-RCELQTPQCDV-----GSTAVVAVVTPEKIIIVSNCGDSR 266

Query: 238 AILVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRLEFPRLKGDLDGQKVL 297
 A+L R PLS + P+ P+ E R+E G +V++
 Sbjct: 267 AVLCRNGVAVPLSSDHKPDV-----PD----ELVRIEAA-----GGRVIY 302

Query: 298 RDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNILKPF 357
 D AR+LG LA+SR +GD+ L KP++
 Sbjct: 303 WD-----GARVLGVLAMSRAIGDNYL-----KPYV 327

Query: 358 LSV PQVTVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRFSLA 417
 +S P+VT++D ED+ +++A+DGLWDV+SN+ +VR L + H S
 Sbjct: 328 ISEPEVTIMD----RSAEDECLILASDGLWDVVSNDTACGVVRMCLRAQKTTSHSESSGR 383

Query: 418 QMLIHSTQ GKEDS 430
 + S + D+
 Sbjct: 384 DAAVRSDKACSDA 396

☐ >gi|21902025|dbj|BAC05575.1| protein phosphatase 2C-like protein [Oryza sativa
 cultivar-group)]
 Length = 467

Score = 81.3 bits (199), Expect = 3e-14

Identities = 82/300 (27%), Positives = 125/300 (41%), Gaps = 95/300 (31%)

Query: 110 LCPEEF-LTGHYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRC 168
 L P F L H++ ++DGHGG A LH+ L +L + +EG V+
 Sbjct: 189 LDPMFSRLPAHFFGVYDGHGGAQVANYCRERLHAALVEEL-SRIEGSVSG----- 237

Query: 169 ICPSDPQFVEEKGIRAEDLVIGALESFAFQECDEVIGRELEASGQM-----GCTALV 220
 ++ VE K E AF+C + E+ + G G TA+V
 Sbjct: 238 ---ANLGSVEFKK-----KWEQAFVDCFSRVDEEVGGNASRGEAVAPETVGSTAVV 285

Query: 221 AVSLQGKLYMANAGDSRAILVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRL 280
 AV + +AN GDSRA+L R + PLS + P E E+ R+
 Sbjct: 286 AVICSSHIIIVANCSDRAVLCRGKQPVPLSVDHKPNRE-----DEYARI 329

Query: 281 EFPRLKGDLDGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLG 340
 E G KV+ Q R+ G LA+SR +G
 Sbjct: 330 EAE-----GGKVI-----QWNGYRVFGLAMSRSIG 355

Query: 341 DHQLRVLDTNILKPFLLSV PQVTVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
 D L KP+++ VP++T++ ++D+ +V+A+DGLWDV+SNE+V + R
 Sbjct: 356 DRYL-----KPWIIPVEITIVP----RAKDDECLVLASDGLWDVMSNEEVCDVAR 402

☐ >gi|15222398|ref|NP_172223.1| protein phosphatase 2C (PP2C), putative [Arabidops
 gi|25352229|pir||B86209 protein F22G5.22 [imported] - Arabidopsis thaliana
 gi|8778547|gb|AAF79555.1| F22G5.22 [Arabidopsis thaliana]
 gi|17979175|gb|AAL49783.1| putative protein phosphatase 2C [Arabidopsis thaliana]

gi|20259129|gb|AAM14280.1| putative phosphatase 2C [Arabidopsis thaliana]
Length = 442

Score = 80.1 bits (196), Expect = 7e-14

Identities = 82/287 (28%), Positives = 120/287 (41%), Gaps = 92/287 (32%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVE 178
HY+ ++DGHG A LH + Q EA+ + + M R D + V

Sbjct: 156 HYFGVYDGHGCSHVAARCKERLHELV--QEEALSDKKEEWKKMME---RSFTRMDKEVV- 209

Query: 179 EKGIRAEDLVIGA---LESAFQECDEVIGRELEASQMGGCTALVAVSLQGKLYMANAGD 235
R + V+ A E +CD V G TA+V+V K+ +AN GD

Sbjct: 210 ----RWGETVMSANCRCELQTPDCDAV-----GSTAVVSVITPEKIIIVANCGD 253

Query: 236 SRAILVRRDEIRPLSFEEFTPE--TERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDLQ 293
SRA+L R + PLS + P+ E RIQ+ G

Sbjct: 254 SRAVLCRNGKAVPLSTDHKKPDRPDELDRIQEA-----GG 287

Query: 294 KVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQL 353
+V++ D AR+LG LA+SR +GD+ L

Sbjct: 288 RVIYWD-----GARVLGVLAMSRAIGDNYL----- 312

Query: 354 KPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
KP++ S P+VTV D +ED+ +++ATDGLWDV++NE +VR

Sbjct: 313 KPYVTSEPEVTVTD----RTEEDEFLILATDGLWDVVTNEAACTMVR 355

gi|8778461|gb|AAF79469.1| F1L3.26 [Arabidopsis thaliana]
Length = 656

Score = 79.3 bits (194), Expect = 1e-13

Identities = 80/310 (25%), Positives = 136/310 (43%), Gaps = 89/310 (28%)

Query: 115 FLTGHYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDP 174
+LT H++ ++DGHGG A + +HS L ++E + E L GR + +

Sbjct: 349 YLTSHFFGVYDGHGGAQVADYCHDRIHSALAEIEIERIKEELCRRNTG---EGRQV-QWEK 404

Query: 175 QFVEEKGIRAEDLVIGAL-ESAFQECDEVIGRELEA-SGQMGGCTALVAVSLQGKLYMAN 232
FV+ ++ +D V G + D ++ LEA S + G TA+VA+ + ++N

Sbjct: 405 VFVDCY-LKVDDEVKKGKINRPVVGSSDRMV---LEAVSPETVGSTAVVALVCSSHIIVSN 460

Query: 233 AGDSRAILVRRDEIRPLSFEEFTPETERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDLQ 292
GDSRA+L+R + PLS + P+ E +

Sbjct: 461 CGDSRAVLLRGKDSMPLSVDHKPDREDE----- 488

Query: 293 QKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDT--- 349
Y R+EK+ K +I QG AR+ G LA+SR + ++R++ T

Sbjct: 489 -----YARIEKAGGK--VIQWQG--ARVSGVLAMSRSI---EMRIITTCYS 527

Query: 350 -----NIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVL 390
+ L+PF++ P+VT + +ED+ +++A+DGLWDV+

Sbjct: 528 LCKIMRSPESGFQSASFSGDQYLEPFVIPDPEVTFMP----RAREDECLILASDGLWDVM 583

Query: 391 SNEQVAWLVR 400
SN++ R

Sbjct: 584 SNQEACDFAR 593

☐ >gi|3643082|gb|AAC36697.1| protein phosphatase-2C; PP2C [Mesembryanthemum crysta
Length = 380

Score = 78.6 bits (192), Expect = 2e-13

Identities = 90/336 (26%), Positives = 143/336 (42%), Gaps = 98/336 (29%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVE 178
H++ ++DGHGG A A +H + + EG+ A

Sbjct: 134 HFFGVYDGHGGSQVAGFCAQRMHEIIAAEWNQ--EGIDAY----- 171

Query: 179 EKGIRAEDLVIGALESFAEQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRA 238
E R ++ I S F+ D+ I E+ AS +M G TA+VAV ++ ++N GDSRA

Sbjct: 172 EWQKRWKEAFI----SGFKRADDQITTEVIAS-EMVGSTAVVAVVSGCQIILSNCGDSRA 226

Query: 239 ILVRRDEIRPLSFEEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKVLFR 298
+L RR + PL+ + P+ E ELL R++G G +V+

Sbjct: 227 VLCRRQTQTIPLTVDHKPDRE-----DELL-----RIEQ--GGRVI-- 260

Query: 299 DHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNILKPFLL 358
W+ AR+ G LA+SR +GD + PF++

Sbjct: 261 -----NWN-----GARVFGVLAMSRAIGDRYM-----SPFII 287

Query: 359 SVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRFSKLAQ 418
VP+VT ED+ +++A+DGLWDV+SN++ + + R +

Sbjct: 288 PVPEVTF----TTRSEDEDECLILASDGLWDVISNDEAGEV-----ARRLLRRRRRAMVA 337

Query: 419 MLIHSTQGKEDSLTEE--GQVSYDDVSFVIPLHSQ 452

I Q D LT+ G+ S D++SV V+ L S+

Sbjct: 338 GDICPAQVVADKLTQLAIGRNSSDNISVIVVDLKSR 373

☐ >gi|15229745|ref|NP_187748.1| protein phosphatase 2C (PP2C), putative [Arabidops
gi|1352681|sp|P49598|P2C4_ARATH Protein phosphatase 2C (PP2C)
gi|1076391|pir||S55457 phosphoprotein phosphatase (EC 3.1.3.16) 2C - Arabidopsis
gi|633028|dbj|BAA07287.1| protein phosphatase 2C [Arabidopsis thaliana]
gi|12322910|gb|AAG51448.1| protein phosphatase 2C (PP2C); 28184-26716 [Arabidopsi
gi|18389242|gb|AAL67064.1| putative protein phosphatase PP2C [Arabidopsis thalian
gi|20259229|gb|AAM14330.1| putative protein phosphatase 2C (PP2C) [Arabidopsis th
Length = 399

Score = 78.6 bits (192), Expect = 2e-13

Identities = 81/296 (27%), Positives = 124/296 (41%), Gaps = 92/296 (31%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSCLRRLQLEAV-----VEGLVATQPPMHLNGRCICPSD 173
H++ +FDGHG A LH +++++E + E +V + M

Sbjct: 136 HFYGVFDGHGCSHVAEKCRERLHDIVKKEVEVMASDEWTETMVKSFKMD----- 185

Query: 174 PQFVEEKGIRAEDLVI-GALESFAEQECD-EVIGRELEASGQMGGCTALVAVSLQGKLYMA 231
+E R +LV+ GA S C E+ + +A G TA+V+V K+ ++

Sbjct: 186 ----KEVSQRECNLVVNGATRSMKNSCRCELQSPQCDAVGS----TAVVSVVTPEKIIVS 237

Query: 232 NAGDSRAILVRRDEIRPLSFEEFTPETTERQRIQQALAFVYPELLAGEFTRLEFPRLKGD 289
N GDSRA+L R PLS + P+ E RIQQ

Sbjct: 238 NCGDSRAVLCRNGVAIPLSVDHKKPDPRDELIRIQA----- 273

Query: 290 DLGQKVLFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD 349
G +V++ D AR+LG LA+SR +GD+ L

Sbjct: 274 --GGRVIYWD-----GARVLGVLAMSRAIGDNYL----- 300

Query: 350 NIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPG 405
 KP+++ P+VTV D ED+ +++A+DGLWDV+ NE + R L G
 Sbjct: 301 ----KPYVIPDPEVTVTD----RTDEDECLILASDGLWDVVPNETACGVARMCLRG 348

☐ >gi|609658|gb|AAA67321.1| protein phosphatase 2C (ptc3+)
 Length = 414

Score = 77.0 bits (188), Expect = 7e-13
 Identities = 84/341 (24%), Positives = 138/341 (40%), Gaps = 104/341 (30%)

Query: 120 YWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEE 179
 ++A++DGHGG A + L L + +P F +

Sbjct: 57 FFAVYDGHGGDKVAKWCGSNLPQILEK-----NPDF--Q 88

Query: 180 KGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAI 239
 KG D V AL+S+F D+ I + + GCTA V + + KLY ANAGDSR +

Sbjct: 89 KG----DFV-NALKSSFLNADKAILDDDDQFHTDPSGCTATVVLRVGNKLYCANAGDSRTV 143

Query: 240 LVRRDEIRPLSFEEFTPETERQRIQQLAFVYPELLAGEFTRLEFPRLKGGDDLQKVLFRD 299
 L + +PLS + P E ++ + A AG F D G

Sbjct: 144 LGSKGIAKPLSADHKPSNEAEKARICA-----AGGFV-----DFG----- 178

Query: 300 HHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKP---F 356
 R+ G LA+SR +GD + TN L+P

Sbjct: 179 -----RVNGNLALSRAIGDFEF----TNSNLEPEKQI 206

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRFSL 416
 + ++P V V ++ +D+ VV+A DG+WD +++QV VR + K+

Sbjct: 207 VTALPDVVVHEIT----DDDEFVVLACDGIWDCKTSQQVIEFVRRGIVAGTS----LEKI 258

Query: 417 AQMLIHSTQKGEDSLTEEGQVSYDDVSVFVIPLHSQGGQESS 457

A+ L+ + + TE + D+++V ++ L + +S+

Sbjct: 259 AENLMDNCIASD---TETTLGLGCDNMTVCIVALLQENDKSA 296

☐ >gi|16226419|gb|AAL16163.1| AT5g59220/mnc17_110 [Arabidopsis thaliana]
 Length = 413

Score = 76.6 bits (187), Expect = 1e-12
 Identities = 79/289 (27%), Positives = 115/289 (39%), Gaps = 89/289 (30%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVE 178
 HY ++DGHG A+ LH +R + EA + + R D + V

Sbjct: 147 HYCGVYDGHGCSHVAMKCRERLHELVEEFADADWEKSM-----ARSFTRMDMEVV- 198

Query: 179 EKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRA 238
 + A+ E +CD V G TA+V+V K+ +AN GDSRA

Sbjct: 199 --ALNADGAAKRCCELQRPDCDAV-----GSTAVVSVLTPEKIIIVANCGDSRA 244

Query: 239 ILVRRDEIRPLSFEEFTPTE--TERQRIQQLAFVYPELLAGEFTRLEFPRLKGGDDLQKVL 296
 +L R + LS + P+ E RIQ G +V+

Sbjct: 245 VLCRNGKAIALSSDHKPDPRDELDRIQAA-----GGRVI 278

Query: 297 FRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKPF 356
 + D R+LG LA+SR +GD+ L KP+

Sbjct: 279 YWD-----GPRVLGVLAMSRAIGDNYL-----KPY 303

Query: 357 LLSVPQVTVLVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPG 405
 ++S P+VTV D DD +++A+DGLWDV+SNE +VR L G

Sbjct: 304 VISRPEVTVTD----RANGDDFLILASDGLWDVVSNETACSVVRMCLRG 348

☐ >gi|15237839|ref|NP_200730.1| protein phosphatase 2C (PP2C), putative [Arabidops
 gi|9759243|dbj|BAB09767.1| contains similarity to Ca/calmodulin-dependent protein
 phosphatase~gene_id:MNC17.13 [Arabidopsis thaliana]
 gi|15809792|gb|AAL06824.1| AT5g59220/mnc17_110 [Arabidopsis thaliana]
 gi|18377817|gb|AAL67095.1| AT5g59220/mnc17_110 [Arabidopsis thaliana]
 Length = 413

Score = 76.6 bits (187), Expect = 1e-12

Identities = 79/289 (27%), Positives = 115/289 (39%), Gaps = 89/289 (30%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVE 178
 HY ++DGHG A+ LH +R + EA + + R D + V

Sbjct: 147 HYCGVYDGHGCSHVAMKCRERLHELVRREEFEADADWEKSM-----ARSFTRMDMEVV- 198

Query: 179 EKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGCTALVAVSLQGKLYMANAGDSRA 238
 + A+ E +CD V G TA+V+V K+ +AN GDSRA

Sbjct: 199 --ALNADGAACKRCELQRPDCDAV-----GSTAVVSVLTPEKIIIVANCGDSRA 244

Query: 239 ILVRRDEIRPLSFETPE--TERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDLQKVL 296
 +L R + LS + P+ E RIQ G +V+

Sbjct: 245 VLCRNGKAIALSSDHKPDPRDELDRIQAA-----GGRVI 278

Query: 297 FRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNILKPF 356
 + D R+LG LA+SR +GD+ L KP+

Sbjct: 279 YWD-----GPRVLGVLAMSRAIGDNYL-----KPY 303

Query: 357 LLSVPQVTVLVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPG 405
 ++S P+VTV D DD +++A+DGLWDV+SNE +VR L G

Sbjct: 304 VISRPEVTVTD----RANGDDFLILASDGLWDVVSNETACSVVRMCLRG 348

☐ >gi|15236110|ref|NP_194338.1| protein phosphatase ABI1 [Arabidopsis thaliana]
 gi|21431817|sp|P49597|P2C1_ARATH Protein phosphatase 2C ABI1 (PP2C) (Absciscic aci
 gi|7488009|pir|T04263 phosphoprotein phosphatase (EC 3.1.3.16) ABI1 - Arabidopsi
 thaliana
 gi|499301|emb|CAA54383.1| ABI1 [Arabidopsis thaliana]
 gi|549981|gb|AAA50237.1| abscisic acid insensitive protein
 gi|4538937|emb|CAB39673.1| protein phosphatase ABI1 [Arabidopsis thaliana]
 gi|7269459|emb|CAB79463.1| protein phosphatase ABI1 [Arabidopsis thaliana]
 gi|23297060|gb|AAN13081.1| phosphatase ABI1 [Arabidopsis thaliana]
 Length = 434

Score = 76.3 bits (186), Expect = 1e-12

Identities = 73/284 (25%), Positives = 118/284 (41%), Gaps = 91/284 (32%)

Query: 118 GHYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFV 177
 H++ ++DGHGG A +H L + +A + PM +C D

Sbjct: 170 AHFFGVYDGHGGSQVANYCRMHLALAE-----IAKEKPM-----LCDGDTWLE 215

Query: 178 EEKGIRAEDLVIGALESFAFQECDEVIGRELEA-SGQMGCTALVAVSLQGKLYMANAGDS 236

```

      + K          AL ++F   D      E+E+ + +   G T++VAV      +++AN GDS
Sbjct: 216 KWKK-----ALFNSFLRVDS----EIESVAPETVGSTSVVAVVFP SHIFVANCGDS 262

Query: 237 RAILVRRDEIRPLSF EFTPETERQRIQQ LAFVYPELLAGEFTRLEFP RRLKGDDL GQKVL 296
      RA+L R      PLS +   P+ E                      E   R+E          G KV+
Sbjct: 263 RAVLCRGKTALPLSVDHKPDRE-----DEAARIEAA-----GGKVI 298

Query: 297 FRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TN IQLKPF 356
      Q   AR+ G LA+SR +GD L          KP
Sbjct: 299 -----QWNGARVFGVLAMSR SIGDRYL-----KPS 323

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
      ++ P+VT +      ++EDD +++A+DG+WDV+++E+   + R
Sbjct: 324 IIPDPEVTAVK----RVKEDDCLILASDGVDVMTDEEACEMAR 363

```

☐ >gi|509419|emb|CAA55484.1| ABI1 [Arabidopsis thaliana]
Length = 434

Score = 76.3 bits (186), Expect = 1e-12
Identities = 73/284 (25%), Positives = 118/284 (41%), Gaps = 91/284 (32%)

```

Query: 118 GHYWALFDGHGGPAAAILAANTLHSC LRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFV 177
      H++ ++DGHGG   A      +H L +      +A + PM      +C D
Sbjct: 170 AHFFGVYDGHGGSQVANYCRERMHLALAE-----IAKEKPM-----LCDGDTWLE 215

Query: 178 EEKGIRAEDLVIGALES AFQECDEVIGRELEA-SGQMGGCTALVAVSLQGKLYMANAGDS 236
      + K          AL ++F   D      E+E+ + +   G T++VAV      +++AN GDS
Sbjct: 216 KWKK-----ALFNSFLRVDS----EIESVAPETVGSTSVVAVVFP SHIFVANCGDS 262

Query: 237 RAILVRRDEIRPLSF EFTPETERQRIQQ LAFVYPELLAGEFTRLEFP RRLKGDDL GQKVL 296
      RA+L R      PLS +   P+ E                      E   R+E          G KV+
Sbjct: 263 RAVLCRGKTALPLSVDHKPDRE-----DEAARIEAA-----GGKVI 298

Query: 297 FRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLD TN IQLKPF 356
      Q   AR+ G LA+SR +GD L          KP
Sbjct: 299 -----QWNGARVFGVLAMSR SIGDRYL-----KPS 323

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
      ++ P+VT +      ++EDD +++A+DG+WDV+++E+   + R
Sbjct: 324 IIPDPEVTAVK----RVKEDDCLILASDGVDVMTDEEACEMAR 363

```

☐ >gi|10432446|emb|CAC10358.1| protein phosphatase 2C [Nicotiana tabacum]
gi|22553023|emb|CAC84141.2| protein phosphatase 2C [Nicotiana tabacum]
Length = 416

Score = 76.3 bits (186), Expect = 1e-12
Identities = 84/346 (24%), Positives = 138/346 (39%), Gaps = 100/346 (28%)

```

Query: 119 HYWALFDGHGGPAAAILAANTLHSC LRRQLEA--VVEGLVATQPPMHLNGRCICPSDPQF 176
      H++ ++DGHG   A+   + +H ++ ++E          V TQ   ++   + S
Sbjct: 152 HFYGVYDGHGCSHVAMKCKDRMHEIVKNEVEKGETQWKEVMTQSFSQMDNEVVHYSS--- 208

Query: 177 VEEKGIRAEDLVIGALES AFQECD-EVIGRELEASGQMGGCTALVAVSLQGKLYMANAGD 235
      GAL +   C E+   + +A G      TA+VAV   K+ ++N GD
Sbjct: 209 -----GALGGSRSNCRCELQTPQCD AVGS-----TAVVAVVTPEKIIVSNCGD 251

```

Query: 236 SRAILVRRDEIRPLSFEEFTPE--TERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQ 293
 SRA+L R PLS + P+ E RIQ+ G
 Sbjct: 252 SRAVLCRNGVAIPLSIDHKPDRPDELNRIQEA-----GG 285

Query: 294 KVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDTNQL 353
 +V++ D R+LG LA+SR +GD+ L
 Sbjct: 286 RVIYWD-----GPRVLGVLAMSRAIGDNYL----- 310

Query: 354 KPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRF 413
 KP+++S P+VT+ + +ED+ +++A+DGLWDV+SNE + R L +
 Sbjct: 311 KPYVISEPEVTITE----RTEEDECLILASDGLWDVVSNETACGVARMCLQSRKPPSPHG 366

Query: 414 SKLAQMLIHSTQ GKEDSLTEEGQV-----SYDDVSVFVIPL 449
 S + + D L + + S D+VSV V+ L
 Sbjct: 367 SPENDVTVTGAGESSDQLCSDASILLTKLALARHSTDNVSVVVVDL 412

☐ >[gi|14334800|gb|AAK59578.1|](#) putative protein phosphatase ABI1 [Arabidopsis thali:
 Length = 434

Score = 76.3 bits (186), Expect = 1e-12
 Identities = 73/284 (25%), Positives = 118/284 (41%), Gaps = 91/284 (32%)

Query: 118 GHYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFV 177
 H++ ++DGHGG A +H L + +A + PM +C D
 Sbjct: 170 AHFFGVYDGHGGSQVANYCRERMHLALAE-----IAKEKPM-----LCDGDTWLE 215

Query: 178 EEKGIRAEDLVIGALESFAFQECDEVIGRELEA-SGQMGGCTALVAVSLQGKLYMANAGDS 236
 + K AL ++F D E+E+ + + G T++VAV +++AN GDS
 Sbjct: 216 KWKK-----ALFNSFLRVDS----EIESVAPETVGSTSVVAVVFP SHIFVANC GDS 262

Query: 237 RAILVRRDEIRPLSFEEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKVL 296
 RA+L R PLS + P+ E E R+E G KV+
 Sbjct: 263 RAVLCRGKTALPLSVDHKPDRE-----DEAARIEAA-----GGKVI 298

Query: 297 FRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDTNQLKPF 356
 Q AR+ G LA+SR +GD L KP
 Sbjct: 299 -----QWNGARVFGVLAMSR SIGDRYL-----KPS 323

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
 ++ P+VT + ++EDD +++A+DG+WDV+++E+ + R
 Sbjct: 324 IIPDPEVTAVK----RVKEDDCLILASDGVDVMTDEEACEMAR 363

☐ >[gi|19113999|ref|NP_593087.1|](#) protein phosphatase 2c homolog 3 [Schizosaccharomyces
[gi|1171964|sp|Q09173|P2C3_SCHPO](#) Protein phosphatase 2C homolog 3 (PP2C-3)
[gi|2130393|pir||S62462](#) protein phosphatase 2c homolog 3 - fission yeast
 (Schizosaccharomyces pombe)
[gi|1019405|emb|CAA91172.1|](#) ptc3 [Schizosaccharomyces pombe]
 Length = 414

Score = 75.9 bits (185), Expect = 1e-12
 Identities = 82/339 (24%), Positives = 140/339 (41%), Gaps = 100/339 (29%)

Query: 120 YWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEE 179
 ++A++DGHGG A + L L + +P F +

Sbjct: 57 FFAVYDGHGGDKVAKWCGSNLPQILEK-----NPDF--Q 88

Query: 180 KGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAI 239
 KG D V AL+S+F D+ I + + GCTA V + + KLY ANAGDSR +

Sbjct: 89 KG----DFV-NALKSSFLNADKAILDDQFHTDPSGCTATVVLRVGNKLYCANAGDSRTV 143

Query: 240 LVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRD 299
 L + +PLS + P E ++ + A AG F D G

Sbjct: 144 LGSKGIAKPLSADHKPSNEAEKARICA-----AGGFV-----DFG----- 178

Query: 300 HHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNIQ-LKPFLL 358
 R+ G LA+SR +GD + + ++N++ K +

Sbjct: 179 -----RVNGNLALSRAIGDFEFK--NSNLEPEKQIVT 208

Query: 359 SVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRFSLAQ 418
 ++P V V ++ +D+ VV+A DG+WD +++QV VR + K+A+

Sbjct: 209 ALPDVVVHEIT----DDDEFVVLACDGIWDCKTSQQVIEFVRRGIVAGTS----LEKIAE 260

Query: 419 MLIHSTQKEDSLTEEGQVSYDDVSFVIPLHSQGQESS 457
 L+ + + TE + D+++V ++ L + +S+

Sbjct: 261 NLMDNCIASD---TETTGLGCDNMTVCIVALLQENDKSA 296

☐ >gi|15227078|ref|NP_180499.1| protein phosphatase 2C (PP2C), putative [Arabidops
 gi|25352214|pir||F84695 probable protein phosphatase 2C [imported] - Arabidopsis
 gi|3980397|gb|AAC95200.1| putative protein phosphatase 2C [Arabidopsis thaliana]
 Length = 362

Score = 75.5 bits (184), Expect = 2e-12

Identities = 80/299 (26%), Positives = 123/299 (41%), Gaps = 90/299 (30%)

Query: 119 HYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVE 178
 HY+ ++DGHG A LH ++ +L + +E + M R D + V

Sbjct: 111 HYFGVYDGHGCSHVAARCRERLHKLQVEELSSDMEDEEEWKTTME---RSFTRMDKEVVS 167

Query: 179 EKGIRAEDLVIGALESFAFQE--CDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDS 236
 + +V + Q CD V G TA+V+V K+ +AN GDS

Sbjct: 168 ----WGDSVVTANCKCDLQTPACDSV-----GSTAVVSVITPDKIVVANCGDS 211

Query: 237 RAILVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRLEFPRLKGGDLGQKVL 296
 RA+L R + PLS + P+ P+ E R+E G +V+

Sbjct: 212 RAVLCRNGKPVPLSTDHKPDR-----PD-----ELDRIE-----GAGGRVI 247

Query: 297 FRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNIQKPF 356
 + D R+LG LA+SR +GD+ L KP+

Sbjct: 248 YWD-----CPRVLGVLAMSRAIGDNYL-----KPY 272

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPG-----NQEDP 410
 + P+VT+ D ++DD +++A+DGLWDV+SNE + R L G + EDP

Sbjct: 273 VSCEPEVTITD-----RRDDDCILIASDGLWDVVSNETACSVARMCLRGGGRRQDNEDP 326

☐ >gi|38109901|gb|EAA55700.1| hypothetical protein MG01351.4 [Magnaporthe grisea]
 Length = 451

Score = 75.5 bits (184), Expect = 2e-12

Identities = 78/339 (23%), Positives = 137/339 (40%), Gaps = 119/339 (35%)

Query: 120 YWALFDGHGGPAAAILAANTLHSCLRRO-----LEAVVEGLVATQPPMHLNGRCICP 171
 ++ +FDGHGG A+ + + +H ++ Q +A+ +G +AT R I
 Sbjct: 63 FFGVFDGHGGDKVALFSGDNIHKIVQNQDTFKSGNIEQALKDGFLLTD-----RAIL- 114

Query: 172 SDPQFVEEKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMA 231
 +DP++ +E + GCTA V + K+Y+A
 Sbjct: 115 NDPKYEDE-----VSGCTACVGLLTDDKIYIA 141

Query: 232 NAGDSRAILVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRLEFPRLKGDLD 291
 NAGDSR++L + +PLSF+ P+ E ++ + A AG F D
 Sbjct: 142 NAGDSRSVLGVKGRAPLSFDHQPNEGEKARITA-----AGGFV-----DF 183

Query: 292 GQKVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDNI 351
 G R+ G LA+SR +GD + + +
 Sbjct: 184 G-----RVNGNLALSRAIGDFEFK---KSA 205

Query: 352 QLKPF---FLLSVPQVTVLVDVQLELQEDDVVVMATDGLWDVLSNEQVAVLVRSLPGNQE 408
 +L P + + P V V D+ +D+ +V+A DG+WD S++ V VR + Q
 Sbjct: 206 ELAPEQQIVTAYPDVVVHDMG----DDDEFVLVIACDGIWDCQSSQAVVEFVRRGIAAKQA 261

Query: 409 DPHRFSKLAQMLIHSTQGKEDSLTEEGQVSYDDVSVFVI 447
 K+ + ++ + S +E G V D++++ +I
 Sbjct: 262 ----LEKICENMDNCLA---SNSETGGVGCDNMTMIII 293

☐ >gi|15242022|ref|NP_200515.1| protein phosphatase 2C, ABI2 [Arabidopsis thaliana]
 gi|3914239|sp|O04719|P2C2_ARATH Protein phosphatase 2C ABI2 (PP2C) (Abscissic acid
 gi|1945140|emb|CAA70163.1| ABI2 protein phosphatase 2C [Arabidopsis thaliana]
 gi|1945142|emb|CAA70162.1| ABI2 protein phosphatase 2C [Arabidopsis thaliana]
 gi|2564213|emb|CAA72538.1| ABI2 [Arabidopsis thaliana]
 gi|8777445|dbj|BAA97035.1| protein phosphatase 2C ABI2 (PP2C) [Arabidopsis thaliana]
 gi|22531154|gb|AAM97081.1| protein phosphatase 2C ABI2 [Arabidopsis thaliana]
 gi|31711886|gb|AAP68299.1| At5g57050 [Arabidopsis thaliana]
 Length = 423

Score = 74.7 bits (182), Expect = 3e-12

Identities = 77/285 (27%), Positives = 121/285 (42%), Gaps = 87/285 (30%)

Query: 116 LTGHYWLFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSPDQ 175
 L+ H++ ++DGHGG A + C R A+ E +V +P C D
 Sbjct: 156 LSAHFFGVYDGHGGSQVA-----NYCRERMHLALTEEIVKEKPEF-----CDGDTW 201

Query: 176 FVEEKGIRAEDLVIGALESFAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGD 235
 +EK +A L ++F D I + + + G T++VAV +++AN GD
 Sbjct: 202 --QEKWKKA-----LFNSFMRVDSEI-ETVAHAPETVGSTSVVAVVFPTHIFVANCGD 251

Query: 236 SRAILVRRDEIRPLSFEEFTPETERQRIQQALFVYPELLAGEFTRLEFPRLKGDLDGQKV 295
 SRA+L R LS + P+ + E R+E G KV
 Sbjct: 252 SRAVLCRGKTPALSLVDHKKPDRD-----DEAARIEAA-----GGKV 287

Query: 296 LFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDNIQLKP 355
 + W+ AR+ G LA+SR +GD L KP
 Sbjct: 288 I-----RWN-----GARVFGVLAMSRSIGDRL-----KP 312

Query: 356 FLLSVPQVTVLVDVQLELQEDDVVVMATDGLWDVLSNEQVAVLVR 400
 ++ P+VT + ++EDD +++A+DGLWDV++NE+V L R
 Sbjct: 313 SVIPDPEVTSVR----RVKEDDCLILASDGLWDVMTNEEVCDLAR 353

☐ >gi|32406686|ref|XP_323956.1| hypothetical protein [Neurospora crassa]
 gi|28920231|gb|EAA29607.1| hypothetical protein [Neurospora crassa]
 gi|28950040|emb|CAD70795.1| probable protein phosphatase 2C [Neurospora crassa]
 Length = 439

Score = 74.7 bits (182), Expect = 3e-12

Identities = 78/339 (23%), Positives = 137/339 (40%), Gaps = 119/339 (35%)

Query: 120 YWALFDGHGGPAAAILAANTLHSLRRQ-----LEAVVEGLVATQPPMHLNGRCICP 171
 ++ +FDGHGG A+ A +H + +Q +A+ +G +AT R I
 Sbjct: 63 FFGVFDGHGGDKVALFAGANIHDIIAKQDTFKTGNYEQALKDGFATD-----RAIL- 114

 Query: 172 SDPQFVEEKGIRAEDLVIGALESASFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMA 231
 +DP++ EE + GCTA V + K+++A
 Sbjct: 115 NDPKYEEE-----VSGCTACVGLITDEKIFVA 141

 Query: 232 NAGDSRAILVRRDEIRPLSFEEFTPETERQRIQQLAFVYPELLAGEFTRLEFPRLKGDDL 291
 NAGDSR++L + +PLSF+ P+ E ++ + A AG F D
 Sbjct: 142 NAGDSRSVLGVKGRAKPLSFDHKPQNEGEKARITA-----AGGFV-----DF 183

 Query: 292 GQKVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDTNI 351
 G R+ G LA+SR +GD + + +
 Sbjct: 184 G-----RVNGNLALSRAIGDFEFK---KSA 205

 Query: 352 QLKP---FLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPGNQE 408
 +L P + + P V V D+ +D+ +V+A DG+WD S++ V VR + Q+
 Sbjct: 206 ELAPEQQIVTAYPDVMVHDL---ADDDEFLVLACDGIWDCQSSQAVVEFVRRGIAAKQD 261

 Query: 409 DPHRFSLAQMLIHSTQGKEDSLTEEGQVSYYDDVSFVI 447
 K+ + ++ + S +E G V D++++ ++
 Sbjct: 262 ----LDKICENMMDNCLA---SNSETGGVGCDNMTMIIV 293

☐ >gi|25352225|pir||F86206 hypothetical protein [imported] - Arabidopsis thaliana
 gi|8954030|gb|AAF82204.1| Contains similarity to protein phosphatase 2C from Arab
 thaliana gb|AF085279. It contains a protein phosphatase
 2C domain PF|00481
 Length = 405

Score = 74.7 bits (182), Expect = 4e-12

Identities = 98/426 (23%), Positives = 165/426 (38%), Gaps = 112/426 (26%)

Query: 39 GSSSSPGAADASRRPDSRPVRSFARGRTLFPWNAGYAEIINAEEKSEFNEDQAACGKLCIRR 98
 GS S PG+ +RP + + G P + A+ E + C R
 Sbjct: 73 GSDSGPGSILKRKRPTTLDIPVAPVGIAAPISN--ADTPREESRAVEREGDGYSVYCKRG 130

 Query: 99 CEFGAEEEWLTLT-----PEEFLTGHYWALFDGHGGPAAAILAANTLHSLRRQL----- 148
 E+ + + P++ + G ++DGHGGP AA AA L S + ++
 Sbjct: 131 KREAMEDRFSAITNLQGDPKQAIFG----VYDGHGGPTAAEFNAKLNCSNILGEIVGGRN 186

 Query: 149 -----EAVVEGLVATQPPMHLNGRCICPSDPQFVEEKGIRAEDLVIGALESASFQECDEVI 203
 EAV G +AT D +F++EK ++
 Sbjct: 187 ESKIEEAVKRGYLAT-----DSEFLKEKNVK----- 212

 Query: 204 GRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEEFTPETERQRIQ 263
 GG + A+ G L +ANAGD RA+L L+ + P + +R

Sbjct: 213 -----GGSCCVTALISDGNLVVANAGDCRAVLSVGGFAEALTS DHRPSRDDR-- 260

Query: 264 QLAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHMSGWSYKRVEKSDLKYPLIHGQ 323
R+E R+ L ++V R +H Y + L+ +

Sbjct: 261 -----NRIESSVRI----LAKEVTVRFYH----DYMVLITIIILQGGYVDTF 298

Query: 324 GRQARLLGTLAVSRGLGDHQLRVLDTNIIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMAT 383
R+ G+LAVSRG+GD LK +++S P++ +L ++ + + +++A+

Sbjct: 299 NSVWRIQGS LAVSRGIGD-----AHLKQWIISEPEINILRINP----QHEFLILAS 345

Query: 384 DGLWDVLSNEQVAWLVRSLPFGNQEDPHRFSLAQMLIHSTQ GKEDSLTEEGQVSYDDVS 443
DGLWD +SN++ + R F G + +++ D G S DD+S

Sbjct: 346 DGLWDKVS NQEAVDIARPFCKGTDQKRKPLLACKKLV-----DLSVSRG--SLDDIS 395

Query: 444 VFVIPL 449
V +I L

Sbjct: 396 VMLIQL 401

☐ >[gi|17555234|ref|NP_497949.1|](#) **L** protein phosphatase 2C, possibly N-myristoylated
[Caenorhabditis elegans]
[gi|18266883|sp|P49596|P2C2 CAEEL](#) Probable protein phosphatase 2C T23F11.1 (PP2C)
[gi|7508306|pir||T25181](#) hypothetical protein T23F11.1 - Caenorhabditis elegans
[gi|5824637|emb|CAA86456.2|](#) Hypothetical protein T23F11.1 [Caenorhabditis elegans]
Length = 356

Score = 74.3 bits (181), Expect = 4e-12
Identities = 76/295 (25%), Positives = 115/295 (38%), Gaps = 97/295 (32%)

Query: 120 YWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEE 179
++A++DGHGG + + LH + Q E

Sbjct: 54 FFAVYDGHGGS KVSQYSGINLHKKVVAQKEF----- 84

Query: 180 KGIRAEDLVIGALES AFQECDEVIGRELEASQMG GCTALVAVSLQGKLYMANAGDSRAI 239
+E + A+E F E D+ + + E + G TA+V + +G +Y NAGDSRA+

Sbjct: 85 ----SEGNMKEAIEKG FLELDQQMRVDEETKDDVSGTTAVVVLIKEGDVYCGNAGDSRAV 140

Query: 240 LVRRDEIRPLSF EFTP--ETERQRIQQ LAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFR 297
E RPLSF+ P ETE +RI + AG

Sbjct: 141 SSVVGEARPLSFDHKPSHETEARRI-----IAAG----- 169

Query: 298 RDHHMSGW-SYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNIIQLKPF 356
GW + RV G LA+SR LGD + DT +

Sbjct: 170 -----GWVEFN RVN-----GNLALS RALGDFAFKNCDTKPAEEQI 204

Query: 357 LLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPFGNQEDPH 411
+ + P V D+L + + +V+A DG+WDV++N++V VR L + DP

Sbjct: 205 VTAFPDVI---TDKLT-PDHEFIVLACDGIWDVMTNQEVVDFVREKL-AEK RDPQ 254

☐ >[gi|4336436|gb|AAD17805.1|](#) protein phosphatase type 2C [Lotus japonicus]
Length = 282

Score = 71.2 bits (173), Expect = 4e-11
Identities = 79/269 (29%), Positives = 111/269 (41%), Gaps = 94/269 (34%)

Query: 189 IGALES AFQECDEVIGRELEASQMG--GCTALVAVSLQ-GKLYMANAGDSRAILVRRDE 245

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      + A++ A+ + D I   LE SG++G G TA+ A+ +   KL +AN GDSRA+L + E
Sbjct: 101 VEAVKKAYVDTSTI---LEKSGELGRGGSTAVTAILINCQKLVVANLGDSRAVLCKNGE 157

Query: 246 IRPLSFEEFTPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHMSGW 305
      PLS + P TE + I+                               R F      GD
Sbjct: 158 AIPLSVDHEPATESEDIRN-----RGGFVSNFPGD----- 187

Query: 306 SYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNIQLKPFLLSVPQVTV 365
      P + GQ                               LAVSR GD           LK L S P VTV
Sbjct: 188 -----VPRVDGQ-----LAVSRAFGDK-----SLKKHLSSEPHVTV 218

Query: 366 LDVDQLELQEDDV--VVMATDGLWDVLSNEQVAWLVRSLPGNQEDPHRFSKLAQMLIHS 423
      EL +DD   +++A+DGLW V+SN++   +R      N +D   +K
Sbjct: 219 -----ELIDDDAEFIILASDGLWKVMSNQEAVDAIR-----NVKDARSAAK----- 259

Query: 424 TQGKEDSLTEEG--QVSYDDVSVFVIPLH 450
      +LTEE   + S DD+S V+ L
Sbjct: 260 -----NLTEEALKRNSDDDISCVVRLQ 282

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☐ >gi|32492311|emb|CAE03844.1| OSJNBb0089K06.2 [Oryza sativa (japonica cultivar-gi
Length = 484

Score = 70.1 bits (170), Expect = 9e-11

Identities = 99/374 (26%), Positives = 151/374 (40%), Gaps = 51/374 (13%)

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Query: 39  GSSSSPGAADASRRPDSRPVRSPARGRTLPNWAGYAEIINAEEKSEFNEDQAACGKLCIRR 98
      G SS+ GAA +RR   P+ S A       W   +   + A +   ED +       R
Sbjct: 85  GCSSTAGAA--ARR---LPLPSGASTAAAVWPVAFGSVSLAGRMRDMEDAVSL-----R 133

Query: 99  CEFGAEEEWLTLCPPEEFLTGHYWALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVAT 158
      F       WL   P       H++A+FDGHGGP + L   +H +   ++ A   L
Sbjct: 134 PSFCT---WLDGSPM-----HFFAVFDGHGGPHVSALCREQMHVIVAEEMVAEAAALRQR 185

Query: 159 QPPMHLNRCICPSDPQFVEEKGIRAEDLVIGALESQECDEVIGRELEASGQMG---G 215
      QP                               V E                               G       SGQ G   G
Sbjct: 186 QPAAMEEEEEERAVAGGAVAE----LRPGGRAGGGGVRVRARHRAGVPCPLSGQTGAIIIG 241

Query: 216 CTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEEFTPETERQRIQQALAFVYPELLAG 275
      TA+VA+ ++ +L ++N GDSRA+L R + PLS +                               L+
Sbjct: 242 STAVVALLVRDRLVVSNCGDSRAVLCRAGDPLPLSSDHKGLNPS-----LSW 288

Query: 276 EFTRLEFPRLKGGDLGQKVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQA-----RLL 330
      TR+   R   GD   GQ V       +SG ++   ++ D K +       GR       R+
Sbjct: 289 RGTRVALARGTWGDKTGQSVGPAALLLSGGAHP--DRPDEKARIEAVGGRVVYLNPRVR 346

Query: 331 GTLAVSRGLGD-HQLRVLDTNIQLKPFLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDV 389
      G LA+SR L +   L       + LKP ++ P +T+       +   +D+ +++A+DG+WDV
Sbjct: 347 GILAMSRALAEGSSLLGPKGDKYLKPEVICEPDITI----TVRTVDDECLILASDGMWDV 402

Query: 390 LSNEQVAWLVRSL 403
      +SNE + + R L
Sbjct: 403 ISNETASDVARQCL 416

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☐ >gi|39591591|emb|CAE71168.1| Hypothetical protein CBG18025 [Caenorhabditis brig
Length = 352

Score = 69.3 bits (168), Expect = 1e-10

Identities = 63/224 (28%), Positives = 94/224 (41%), Gaps = 64/224 (28%)

Query: 191 ALESAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLS 250
A+E F + D+ + + E + G TA+V + +G +Y NAGDSRA+ E RPLS

Sbjct: 92 AIERGFLDLQOMRVDEETKDDVSGTTAVVVLIEGVDVYCGNAGDSRAVSSVLGEARPLS 151

Query: 251 FEFTP--ETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKVLFRDHHMSGW-SY 307
F+ P E E +RI + AG GW +

Sbjct: 152 FDHKPSHEIEARRI-----IAAG-----GWVEF 174

Query: 308 KRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKPFLLSVPQVTVLD 367
RV G LA+SR LGD + DT + + + P V

Sbjct: 175 NRVN-----GNLALSRLGDTFKNCDTKPAEEQIVTAFDPDVI--- 212

Query: 368 VDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPQGNQEDPH 411
D+L + + +V+A DG+WDV++N++V VR L + DP

Sbjct: 213 TDKLT-PDHEFIVLACDGIWDMTNQEVVDFVREKL-SEKRDPQ 254

☐ >[gi|6478146|emb|CAB61839.1|](#) putative serine/threonine phosphatase type 2c [Sporobolus stapfianus]
Length = 271

Score = 69.3 bits (168), Expect = 1e-10

Identities = 82/338 (24%), Positives = 136/338 (40%), Gaps = 121/338 (35%)

Query: 121 WALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEEK 180
+ +FDGHGG AA AA + P+FV E+

Sbjct: 44 FGVFDGHGGKNAAEFAAENM-----PKFVAEE 70

Query: 181 GIRA---EDLVIGALESFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSR 237
+A E + GA++ + + DE E G+ GG + AV +G L ++N GD R

Sbjct: 71 MTKADGGESEIEGAVKRGYLTDE---EFLRRGESGGACCVTAVLQKGGGLVVSNGVDCR 126

Query: 238 AILVRRDEIRPLSFEF--TPETERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKQV 295
A+L R + L+ + + E E++RI+ +LG V

Sbjct: 127 AVLSRSGKAEALTSDHRSAREDEKERIE-----NLGGFV 160

Query: 296 LFRDHHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNQLKP 355
+ ++ W R+ G+LAVSRG+GD LK

Sbjct: 161 V---NYRGTW-----RVQGS LAVSRGIGD-----AHLKQ 186

Query: 356 FLLSVPQVTVLDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSLPQGNQEDPHRFSK 415
++++ P L VDQ + +++A+DGLWD + N++ L R N + S+

Sbjct: 187 WIVADPDTRTLLVDQ----HCEFLILASDGLWDKIDNQEAVDLARPLCTSND----KASR 238

Query: 416 LA--QMLIHSTQKEDSLTEEGQVSYYDDVSFVIPLHS 451
+A +ML+ ++ + S DD+SV +I L +

Sbjct: 239 MAACRMLVETSISRG-----STDDISVVIIQLQN 267

☐ >[gi|15220548|ref|NP_176948.1|](#) protein phosphatase 2C (PP2C), putative [Arabidopsis thaliana]
[gi|25404969|pir||H96700](#) protein F12A21.5 [imported] - Arabidopsis thaliana
[gi|11072032|gb|AAG28911.1|](#) F12A21.5 [Arabidopsis thaliana]
Length = 464

Score = 68.6 bits (166), Expect = 2e-10

Identities = 69/286 (24%), Positives = 118/286 (41%), Gaps = 90/286 (31%)

Query: 120 YWALFDGHGGPAAAILAANTLHSCLRRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEE 179
 ++ ++DGHGG AA A LH + +E C + VE

Sbjct: 151 FFGVYDGHGGAKAAEFVAENLHKYVEMMEN-----CKGKEEKVE- 190

Query: 180 KGIRAEDLVIGALESFAFQECD-----EVIGRELEASGQMGGCTALVAVSLQGKLYMANAGD 235
 A ++AF D E + +E G + G + AV ++ ++N GD

Sbjct: 191 -----AFKAAFLRTDRDFLEKVIKEQSLKGVVSGACCVTAVIQDQEMIVSNLGD 239

Query: 236 SRAILVRRDEIRPLSFEEFTP--ETERQRIQQLAFVYPELLAGEFTRLEFPRLKGGDLGQ 293
 RA+L R L+ + P + E++RI+ + + P + G L+G

Sbjct: 240 CRAVLCRAGVAEALTDDHKPGRDDEKERIESQSLI-PFMTFG-----LQGG---- 284

Query: 294 KVLFRDHMSGWSYKRVEKSDLKYPLIHGQGRQARLLGTLAVSRGLGDHQLRVLDTNLQ 353
 + D+H W R+ G LAVSR +GD L

Sbjct: 285 ---YVDNHQGAW-----RVQGILAVSRSIGD-----AHL 310

Query: 354 KPFLLSVPQVTVLVDVDQLELQEDDVVVMATDGLWDVLSNEQVAWL 399

K ++++ P+ VL+++Q + + +V+A+DGLWDV+SN++ + V

Sbjct: 311 KKWVVAEPETRVLELEQ----DMEFLVLASDGLWDVVSNEQVAVTV 352

☐ >gi|34908256|ref|NP_915475.1| putative protein phosphatase 2C [Oryza sativa (japonica cultivar-group)]

gi|20160622|dbj|BAB89568.1| putative protein phosphatase 2C [Oryza sativa (japonica cultivar-group)]

Length = 405

Score = 67.8 bits (164), Expect = 4e-10

Identities = 95/382 (24%), Positives = 151/382 (39%), Gaps = 109/382 (28%)

Query: 30 PYRRPRFLRGSSSSPGAADASRRPDSRPV-RSPARGRTL PWNAGYAEI INAEKSEFNEDQ 88
 P +R R R +S +P RPD P+ GRT EF +

Sbjct: 55 PGKRQRLARTASGAP-----RPDEDSASERPSCGRT-----EEF--PR 90

Query: 89 AACGKLCIRRCFEFGAEEEWLTLCP EEF LTG----HYWALFDGHGGPAAAILAANTLHSC 144
 +C RR E E+ +++ P+ FL H++ +FDGHG A + +H +

Sbjct: 91 YGVTAVCGRRREM---EDAVSIRPD-FLPASGKFHFYGVFDGHGCSHVATTCQDRMHEIV 146

Query: 145 RRLQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEEKGIRAEDLVIGALESFAFQECDEVIG 204
 + G VA P + + D + R++D E

Sbjct: 147 AEEHNKGASGEVA--PWRDVMEKSFARM DGEVGNRASTRSDDEPACPCE----- 193

Query: 205 RELEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLSFEEFTPETERQRIQ 264
 ++ + G TA+VAV ++ +ANAGDSRA++ R LS + P+

Sbjct: 194 QQTPSRRDHAGSTAVVAVVSPTQVVVANAGDSRAVISRAGVPVALSVDHKPDR----- 246

Query: 265 LAFVYPELLAGEFTRLEFPRLKGGDLGQKVLFRDHMSGWSYKRVEKSDLKYPLIHGQG 324
 P+ E R+E G +V++ D

Sbjct: 247 -----PD-----ELERIEAA-----GGRVIYWD----- 264

Query: 325 RQARLLGTLAVSRGLGDHQLRVLDTNLQKPFLLSVPQVTVLVDVDQLELQEDDVVVMATD 384
 AR+LG LA+SR +GD L KP++ S P+VTV + +D+ +++A+D

Sbjct: 265 -GARVLGVLAMSRAIGDGYL-----KPYVTSEPEVTVTE----RTDDDECLILASD 310

Query: 385 GLWDVLSNEQVAWLVRSLP 406

GLWDV++NE +VR+ N
 Sbjct: 311 GLWDVVTNEMACEVVRRACFHHN 332

☐ >gi|30693757|ref|NP_175057.2| protein phosphatase 2C (PP2C), putative [Arabidops
 gi|17381034|gb|AAL36329.1| putative protein phosphatase type 2C [Arabidopsis thal
 gi|21436259|gb|AAM51268.1| putative protein phosphatase type 2C [Arabidopsis thal
 Length = 371

Score = 66.6 bits (161), Expect = 8e-10
 Identities = 59/212 (27%), Positives = 86/212 (40%), Gaps = 68/212 (32%)

Query: 191 ALESAFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIRPLS 250
 A+ F++ DE E + G TA A + KL +AN GDSR + R PLS

Sbjct: 191 AIVEVFKQTDEEYLIEEAGQPKNAGSTAATAFLIGDKLIVANVGDSRVVASRNGSAVPLS 250

Query: 251 FEFTPE--TERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKVLFRDHHMSGWSYK 308
 + P+ ERQRI +D G +++ W

Sbjct: 251 DDHKPDRSDERQRI-----EDAGGFIIW----AGTW--- 277

Query: 309 RVEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDTNLQKPFLLSVPQVTVLDV 368
 R+ G LAVSR GD QL KP++++ P++ D+

Sbjct: 278 -----RVGGILAVSRAFGDKQL-----KPYVIAEPEIQEEDI 309

Query: 369 DQLELQEDDVVVMATDGLWDVLSNEQVAWLVR 400
 LE +V+A+DGLW+VLSN+ +VR

Sbjct: 310 STLEF-----IVVASDGLWNVLSNKAIVR 336

☐ >gi|34902050|ref|NP_912371.1| putative Serine/threonine phosphatases [Oryza sat:
 cultivar-group)]
 gi|29893648|gb|AAP06902.1| putative Serine/threonine phosphatases [Oryza sativa (
 cultivar-group)]
 gi|29893658|gb|AAP06912.1| unknown protein [Oryza sativa (japonica cultivar-group)
 Length = 404

Score = 66.6 bits (161), Expect = 1e-09
 Identities = 89/338 (26%), Positives = 130/338 (38%), Gaps = 99/338 (29%)

Query: 78 NAEKSEFNEDQAACGKLCIRRCEFGAEEEWLTLCPPEEFLTGH-YWALFDGHGGPAAAILA 136
 + E E + +C RR + E+ ++ CP FL GH ++ +FDGHG A

Sbjct: 67 DEEDREVERARYGFTSVCGRRRDM---EDSVSACPG-FLPGHHFFGVFDGHGCSHVAT-- 120

Query: 137 ANTLHSLCLRRQLEAVVEGLVATQPPMHLNG-----RCICPSDPQFVEEKGIRAEDL 187
 SC +R E VV+ A L+ R D + V

Sbjct: 121 -----SCGQRMHEIVVDEAGAAAGSAGLDEEARWRGVMERSFARMDAEAVASSRGSVAPA 175

Query: 188 VIGALESFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAILVRRDEIR 247
 E +CD V G TA+VAV + +AN GDSRA+L R

Sbjct: 176 PTCRCQMQLPKCDHV-----GSTAVVAVLGPRHVVVANCGDSRAVLCRGGAII 223

Query: 248 PLSFEFTPE--TERQRIQQALAFVYPELLAGEFTRLEFPRLKGGDDLQKVLFRDHHMSGW 305
 PLS + P+ E +RI G +V+F D

Sbjct: 224 PLSCDHKPDPRDELERIHAA-----GGRVIFWD----- 251

Query: 306 SYKRVEKSDLKYPLIHGQGRQARLLGLTAVSRGLGDHQLRVLDTNLQKPFLLSVPQVTV 365
 AR+ G LA+SR +GD L KP+++ P+V V

Sbjct: 252 -----GARVFGMLAMSRAIGDSYL-----KPYVICDPEVRV 282

Query: 366 LDVDQLELQEDDVVVMATDGLWDVLSNEQVAWLVRSL 403

++ ED+ +++A+DGLWDV+SNE +VR+ L

Sbjct: 283 MERKD---GEDEFLILASDGLWDVVSNEVACNVVRACL 317

☐ >[gi|26331022|dbj|BAC29241.1|](#) ☒ unnamed protein product [Mus musculus]
[gi|26337573|dbj|BAC32472.1|](#) ☒ unnamed protein product [Mus musculus]
 Length = 360

Score = 66.2 bits (160), Expect = 1e-09

Identities = 75/315 (23%), Positives = 122/315 (38%), Gaps = 96/315 (30%)

Query: 121 WALFDGHGGPAAAILAANTLHSLRRQLEAVVEGLVATQPPMHLNGRCICPSDPQFVEEK 180
 + +FDGHGG AA + L L++ L+ D + +E

Sbjct: 124 FGIFDGHGGETA AEYVKSRLPEALKQHLQ-----DYEKDKEN 160

Query: 181 GIRAEDLVIGALES AFQECDEVIGRELEASGQMGGCTALVAVSLQGKLYMANAGDSRAIL 240
 + + LE D + +L S G T L+A+ L +AN GDSR +L

Sbjct: 161 SVLTYQTI---LEQQILSIDREMLEKLTVS YDEAGTTCLIALLSDKDLTVANVGDSRGVL 217

Query: 241 VRRD-EIRPLSF EFTPET--ERQRIQQ LAFVYPELLAGEFTRLEFP RRLKGDDL GQKVLF 297
 +D PLS + P ER+RI++ AG F

Sbjct: 218 CDKDGNAIPLSHDHKPYQLKERKRIKR-----AGGFISFN----- 252

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☐ >[gi|34857062|ref|XP_227247.2|](#) ☒ similar to protein phosphatase 2C epsilon [Rattus
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Alterations in the activity and expression of serine/threonine protein phosphatases during all *trans* retinoic acid-induced apoptosis in breast cancer cells

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Abstract. Retinoids exert different effects on malignant cells with various phenomena. They can induce differentiation and apoptosis in various cancer cells. However, the underlying mechanism of these effects is not clear. There are data related to the role of protein phosphatases during retinoid-induced leukemic cell differentiation. The aim of this study was to evaluate effects of the All *trans* retinoic acid (ATRA) on protein/serine phosphatases during ATRA induced apoptosis in the breast cancer cells. The MTT assay was used to determine drug-mediated cytotoxicity. A cell death detection ELISA kit was used for detection of the DNA fragments. The activity of serine/threonine protein phosphatases was assayed by the serine/threonine phosphatase system. The expression of serine/threonine protein phosphatases was evaluated by Western blot. During ATRA treatment, a significant decrease in the activity of serine/threonine phosphatases 2A, B and C occurred. The decreased activity of PP2A correlated with the up-regulation of PP2A catalytic and PP2A/B γ , PP2A/B α regulatory subunits. The decrease in activity of the PP2B correlated with down-regulation of PP2B catalytic and up-regulation of PP2B regulatory subunit expression. In addition, there was an up-regulation in PP4C and down regulation in PP2C α/β subunits protein expression. We demonstrated clear alteration in the activity and expression of serine/threonine protein phosphatases in breast cancer cells during ATRA treatment, and we suggest that the ATRA-induced apoptosis of the MCF-7 cells is significantly related to the phosphorylation dynamics.

Introduction

All *trans* retinoic acid (ATRA) is being utilized in the treatment of acute promyelocytic leukemia, early stage head and neck, and skin cancer (1-3). They have a regulatory effect on cellular biological phenomena such as growth, development, proliferation and differentiation (4). Retinoids exert their effect at the cellular level via nucleosolic receptors (5-8). RARs, RXRs, estrogen (ER) and vitamin D receptors are members of the steroid-thyroid receptor superfamily (9,10). There is an interaction between ERs and RARs (11,12). All these nucleosolic receptors are molecular targets in the diagnosis, prophylaxis, and treatment of breast cancer (13-15). ATRA selectively inhibits the proliferation of ER positive breast cancer cells (16,17).

Our group has reported the role of serine/threonine protein phosphatases during retinoic-acid-induced leukemic cell differentiation (18).

Serine/threonine protein phosphatases are important enzymes which have the potential for balancing the phosphorylation status of the cell by dephosphorylating critical substrates (19,20). The major serine/threonine protein phosphatase catalytic subunits in mammalian cells comprise four forms which have been designated as protein phosphatase type 1 (PP1), 2 A (PP2A), 2 B (PP2B, calcineurin), and 2 C (PP2C), based on the classification system proposed by Cohen (21,22). The core of PP2A consists of a 36 kDa catalytic subunit (PP2A/C) complexed with a constant regulatory subunit of 65 kDa (PR 65 or A subunit). This core dimer associates with variable regulatory subunits (B subunits). The exact function of the regulatory subunits is not yet completely resolved but they probably influence substrate specificity and subcellular localization (20).

PP2B has been identified in various tissues, and was originally thought to occur primarily in nerve tissue (23). The PP2B holoenzyme purified from mammalian cells is a heterotrimeric complex consisting of a large ~60 kDa catalytic subunit, PP2B/A (CNA), PP2B/B (CNB), and calmodulin, an ~17 kDa Ca⁺⁺-receptive protein (24,25). The A subunit binds calmodulin whereas the B subunit attaches to four molecules of Ca⁺⁺. PP2B regulatory subunit, CNB is the key site of association for both the CsA- and FK506-immunophilin

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complexes (25). PP2B is incapable of functioning as a phosphatase *in vitro* when calmodulin or CNB is absent. Although sequences of CNB and calmodulin are related, neither of the two proteins can replace the other's absence. PP2B is required in the activation and proliferation of the T lymphocytes to activate the NF-AT transcription factor involved in regulation of the interleukin-2 (IL-2) receptor gene and NF- κ B serving as a molecular switch (26). However, the target substrates for the PP2B dephosphorylation which is needed to activate gene expression in T cells, have not been identified. Calmodulin is implicated as the mediator of Ca^{++} -dependent regulation of cell cycle progression, and its concentration increases two-fold at the G1/S boundary (27). Recent studies suggest that PP2B may play a role in early cell cycle events (28,29).

PP2C is present in many tissues at much lower levels than the other types of protein phosphatases (30-32). It has been reported that the expression of the PP2C gene is enhanced during MyoD1-induced myogenic differentiation of embryonic mesenchymal cells (33).

One of the important new members of the PP2 family is PP2X (PP4C). It is a 34 kDa protein whose amino acid sequence shows 65% identity to PP2A and 45% identity to PP1. Immuno-fluorescence studies revealed that PP4C is predominantly associated with centrosomes (34), but its function at this location is, at present, unclear.

The evaluable data demonstrate that there is a significant and biologically relevant interaction between the nucleosolic hormone receptor family and protein phosphatases family. In this study, we investigated the probable relationship of serine/threonine protein phosphatases 2 family and ATRA-induced cytotoxicity/apoptosis of MCF-7 breast cancer cells as a model.

Materials and methods

Tumor cells. The human breast cancer cell line MCF-7 was kindly provided by Dr J.R. Bertino from Memorial Sloan-Kettering Cancer Center, NY, USA. This tumor cell line is maintained in culture as adherent cells and cultured in RPMI-1640 plus 10% heat inactivated fetal calf serum added to 1% L-glutamine, 1% non-essential amino acids, 10,000 units/ml penicillin, and 10 μ g/ml streptomycin. The cell line was grown in a humidified atmosphere at 37°C in 5% CO₂. When the tumor cell lines were used as target cells, they were treated with trypsin-EDTA, washed, and resuspended in the complete medium. All the above material was purchased from Sigma Chemical Co. (St. Louis, MO).

Reagents. ATRA, 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and phosphate-buffered saline (PBS) were purchased from Sigma Chemical Co. The Cell Death Detection ELISA^{plus} kit was purchased from Boehringer Mannheim, Germany. The stock solution of ATRA was prepared in ethanol.

Rabbit anti serine/threonine PP2A antibody to catalytic subunit was obtained from Promega Corporation (Madison, USA) and antibodies to 2A/A (65 kDa), 2A/B α (55 kDa), 2A/B γ (55 kDa) were obtained from Biogenesis Ltd. (Poole, UK).

Anti-PP2B catalytic subunit mouse monoclonal antibody was obtained from Transduction laboratories, Lexington, UK and anti-PP2B regulatory subunit rabbit polyclonal antibody, and anti-calmodulin mouse monoclonal antibody from Biomol, PA. Anti-PP2C catalytic subunit α/β rabbit polyclonal antibody and antiprotein phosphatase PP2X (PP4C) catalytic subunit rabbit polyclonal antibody were purchased from Biogenesis. Monoclonal human Anti-bcl-2 antibody was purchased from R&D systems, Inc.

Cytotoxicity assay. The MTT assay was used to determine drug-mediated cytotoxicity as described previously (35). For MTT assay, briefly, target tumor cells were resuspended in the medium at 1×10^5 cells/ml after verifying cell viability by trypan blue dye exclusion assay (Sigma Chemical Co.) (36). The stock solution of ATRA was prepared as 10^{-2} M. Afterwards 1/10 dilutions were made and the cytotoxicity was determined by trypan blue dye exclusion test and MTT assay. Incubation time of 72 h was used because of the doubling time experiments of the MCF-7 cells beforehand.

Experiments were performed at least three times with the representative data presented.

DNA fragment detection by ELISA. A cell death detection ELISA kit (Cell Death Detection ELISA^{plus}; Boehringer Mannheim, Germany) was used according to the manufacturer's instructions for detection of DNA fragments (37). The principle of this test is based on the detection of mono- and oligo-nucleosomes in the cytoplasmic fractions of cell lysates by using biotinylated antihistone- and peroxidase-coupled anti-DNA antibodies. An enrichment factor is used as a parameter of apoptosis and shown on the Y-axis as mean \pm standard deviation (SD) of triplicates. Assays were performed at least three times, and data shown are representative of those.

Preparation of cytosolic fraction. MCF-7 cells (15×10^6) were washed three times with ice-cold TBS (pH 7.4) and then disrupted in 1 ml of homogenization buffer containing 20 mM Tris-HCl, pH 7.4, 2 mM dithiothreitol, 2 mM EDTA, 2 mM EGTA, 0.25 mM sucrose, protease inhibitors 0.1 mM leupeptin and 0.02 mM TLCK (N α -p Tosyl-L-lysine-chloromethyl-ketone) by a glass to glass Potter Elvehjem homogeniser. The homogenates were immediately centrifuged at 1000 \times g for 10 min and the supernatant was centrifuged at 100 000 \times g for 1 h. The resulting supernatant was used as the cytosolic fraction.

Protein assay. Total protein concentration in the cell culture extracts was measured by the Lowry's method (38). The assay was standardised with bovine serum albumin.

Phosphatase activity. Activities of serine/threonine protein phosphatases were assayed by serine/threonine phosphatase system (Promega, USA) according to manufacturer's instructions. Cell supernatants were extracted from the columns in a PP2A, PP2B, PP2C specific medium. The comparison of PP2 enzyme activities during ATRA-induced apoptosis of the MCF-7 cells with changing concentrations of ATRA, was performed in the cytosolic fractions of MCF-7 cells, after incubation of ATRA for 72 h.

Activity of serine/threonine protein phosphatases was determined after washing of debris and dead cells. The activity was measured by a non-radioactive method based on determination of free phosphate with colour development. After treatment, the polypeptide substrate with the enzyme samples reaction was terminated with Molybdate Dye/Additive mix at the 3rd min and the dye color (ammonium molybdate-malachite green) was allowed to develop for 30 min. The absorbance was read at 630 nm with a plate reader (Coda, Bio-Rad, USA).

Data were analyzed by ANOVA with significance set at P-value of <0.05.

Western blotting method for detecting Bcl-2, PP2 catalytic and regulatory subunits. Cell extract samples incubated by ATRA at 72 h were promptly heated (100°C, 5 min) and analysed by polyacrylamide gel electrophoresis (PAGE, 10% gels), followed by Western blotting onto nitrocellulose membranes using samples of 20 µg protein/well. After blocking with blotto (5% milk, 0.1% Tween-20, 10 mM Tris-HCl, pH 7.5, 150 mM NaCl) for 1 h, the membrane was exposed to the primary antibody according to the manufacturer's instructions. The secondary antibodies were coupled to strepto-avidin-biotinylated alkaline phosphatase complex. Serine/threonine phosphopeptide (BCIP/NBT) substrate was utilized to achieve the color reaction by AP color reactive. The same methods were performed for bcl-2 detection.

Results

Cytotoxic and apoptotic effect of ATRA on MCF-7 cells. The IC₅₀ value was found to be 5×10⁻⁶ M at 72 h incubation by ATRA (Fig. 1). To evaluate probable induction of apoptosis by ATRA, the cell suspensions were treated by changing concentrations of ATRA. Mono- and oligo-nucleosomes were measured. It was shown that the enrichment factor of mono- and oligo-nucleosomes increased in correlation with the increasing concentrations of ATRA (Fig. 2).

Effect of ATRA on serine/threonine protein phosphatase 2 enzyme activities in MCF-7 cell cytosolic fractions. The PP2A protein phosphatase enzyme activity was found to be 1.19 pmol phosphate/min/µg protein in the control cytosolic fraction without ATRA. After treatment with ATRA it showed a gradual decrease in the PP2A activity reaching 0.89 pmol phosphate/min/µg protein with the increasing concentrations of ATRA. The lowest PP2A activity was measured with 10⁻⁵ ATRA. The total decrease of PP2A activity compared with the control was 25.2% (One-way ANOVA demonstrated a significant difference, P-value <0.05) (Fig. 3A).

PP2B activity was measured as 1.77 pmol phosphate/min/µg protein in the control fraction and reached 1.31 pmol phosphate/min/µg protein by 10⁻⁵ M ATRA and a gradual decrease with increasing concentrations of ATRA (One-way ANOVA demonstrated a significant difference, P <0.05) (Fig. 3B). PP2C activity also decreased from 1.16 to 0.89 pmol phosphate/min/µg protein with a total decrease of 23.3% compared with the control (One-way ANOVA demonstrated a significant difference, P <0.05) (Fig. 3C).

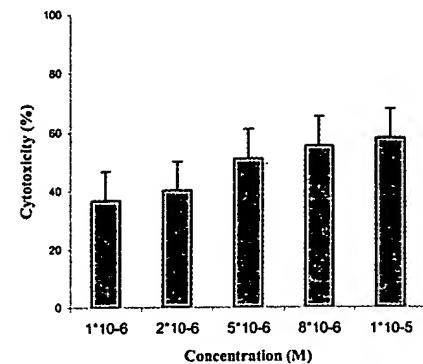


Figure 1. Cytotoxicity of the MCF-7 cells treated with different concentrations of ATRA. The data represent the mean of three different experiments with the SD not exceeding 5%.

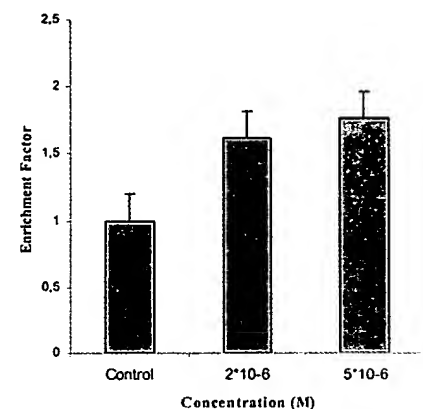


Figure 2. ATRA-induced apoptosis in human breast carcinoma cell line MCF-7. The cells were treated with different concentrations of ATRA. Apoptosis was examined by determining nucleosomal DNA fragmentation using an apoptosis determination kit. Rate of apoptosis is reflected by enrichment of nucleosomes in the cytoplasm shown on the Y-axis. The data represent the mean of three different experiments with the SD not exceeding 5%.

PP2A catalytic and regulatory subunit up-regulation during ATRA-induced apoptosis of the MCF-7 cells. MCF-7 cells were treated with the increased concentrations of ATRA from 10⁻⁶ to 10⁻⁵ M and cytosolic fractions were extracted at 72 h. The extracts were subjected to immunoblot analysis with anti-PP2A antibodies. There was a significant up-regulation of PP2A/C catalytic subunit, and PP2A/B γ and PP2A/B α regulatory subunits. There was no change in the expression of PP2A regulatory A subunit (Fig. 4).

PP2B catalytic subunit down-regulation and regulatory subunit up-regulation during ATRA-induced apoptosis of the MCF-7 cells. The beforementioned cytosolic extracts were treated with anti-PP2B and anti-calmodulin antibodies in the immunoblot system. There appeared to be down-regulation of the PP2B catalytic subunit and up-regulation of the PP2B regulatory subunit. No change occurred with the calmodulin expression (Fig. 4).

PP2C and PP4C expression during ATRA-induced apoptosis of the MCF-7 cells. Down-regulation in the expression of

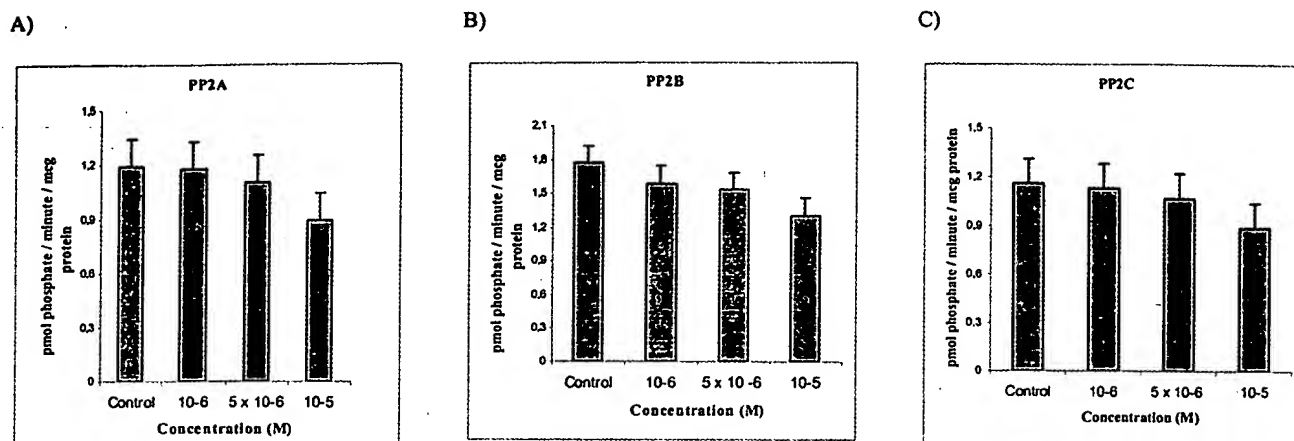


Figure 3. Protein phosphatase activities in cytosolic fractions of the MCF-7 cells with changing concentrations of ATRA. Protein phosphatase activity was measured after the addition of ATRA and compared with control cells. Each point represents the mean value of three separate experiments (SD <5%). After treatment with ATRA it showed a gradual decrease in PP2A (A), PP2B (B), and PP2C activity (C).

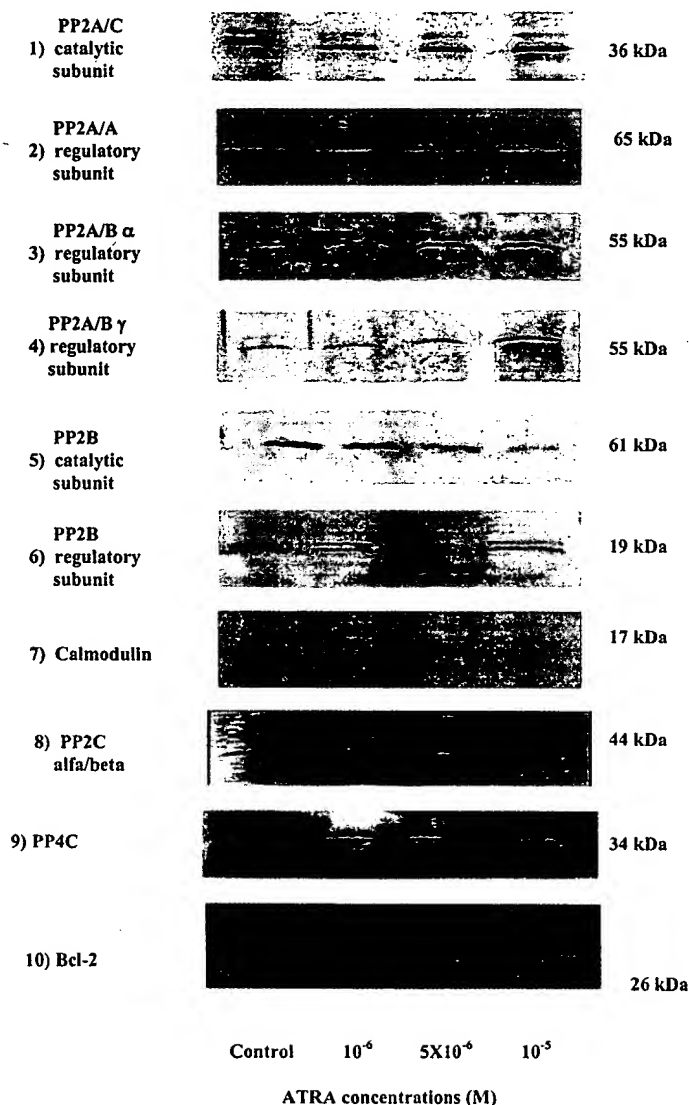


Figure 4. Western analysis of the serine/threonine protein phosphatase and Bcl-2 expression. Homogenates of the MCF-7 cells were fractionated by SDS-PAGE (10% gel, 20 μ g protein/well), blotted onto nitrocellulose membranes and immunoblots with PP2A catalytic and PP2A/A regulatory subunit, PP2A/B α and PP2A/B γ regulatory subunits, PP2B catalytic and regulatory subunit, calmodulin, PP2C α/β , PP4C, and bcl-2. There was a significant up-regulation of PP2A/C catalytic subunit (1), PP2A/B γ (4) and PP2A/B α regulatory subunits (3), PP2B regulatory subunit (6), PP4C (9) and down-regulation of PP2B catalytic subunit (5), and PP2C (8). There was no change in the expression of PP2A/A regulatory subunit (2), calmodulin (7), and bcl-2 expression (10). Each experiment was repeated at least three times and presented with one representative result.

PP2C and up-regulation in PP4C protein expression was observed (Fig. 4).

Bcl-2 expression during ATRA-induced apoptosis of the MCF-7 cells. MCF-7 cells were cultured for 72 h in the presence of ATRA. Protein extracts were prepared and Western blot analysis was performed. As shown in Fig. 4, after administration of ATRA no alteration in the bcl-2 protein expression was detected.

Discussion

There is a need for new treatments of breast cancer, which cannot be cured with the conventional therapeutic modalities. There is also a requirement for an increase of the survival advantage in many late stage patients. Therefore, new molecular targets and new agents are essential.

The induction of apoptosis with nucleo-hormone ligands is given great importance in cancer therapy. Interactions among the nuclear receptor family are reported (11,12). It has been demonstrated that there is a positive interaction between ER and RAR and also that ER positive breast cancer cells express more RAR α -receptors than ER negative cells (11,12). In addition, it was shown that retinoic acid induces ER expression in the MCF-7 cells (39). ATRA is reported to induce cytotoxicity and apoptosis in the hormone receptor positive breast cancer cell lines (40-42). However, the mechanisms underlying the ATRA-induced cytotoxicity and apoptosis are unclear. ATRA exerts its effect by binding to the RAR α -receptors in the nucleosol. These receptors function as transcription factors when bound to ATRA. It is known that the ATRA-RAR α complex affects critical sequences related to G1-S check point and suppresses proliferation (41). In the leukemic cell lines, ATRA induced bcl-2 down-regulation and resulting apoptosis was demonstrated (43,44).

The activation and inactivation of critical substrates related to apoptosis are significantly affected by the phosphorylation status. The phosphatases, which are responsible for dephosphorylation, are activated in contrast to kinases and have an important role in the proliferation-apoptosis uncoupling. Our group has demonstrated a significant relationship between the PP2A expression and leukemic cell differentiation in the HLA 60 and K562 myeloid leukemia cell lines (45,46). We demonstrated PP2A catalytic subunit down-regulation during ATRA-induced leukemic differentiation. Ratnasinghe *et al* reported a decrease in the PP2A expression and activity in adriamycin resistant MCF-7 cells (47). Gopalakrishna *et al* reported that the PP2A activity is higher in ER positive MCF-7 than in ER negative MCF-7 cell lines, indicating that PP2A has a role in the interaction of MCF-7 and drug-induced apoptosis (48).

In this study, we demonstrated that there was a clear alteration in the activity and expression of the serine/threonine protein phosphatases in breast cancer cells during ATRA treatment. Our findings show a clear-cut decrease in the activity of the PP2A together with B regulatory subunit up-regulation. In addition, we found a decrease in PP2B activity in concordance with PP2B catalytic subunit down-regulation, whereas, the PP2C activity was decreased and the PP4C expression increased. Altogether we suggest that the PP2

family strongly interacts with the critical substrates related to ATRA-induced apoptosis.

Finally, we have investigated the expression of the bcl-2 which is thought to protect against the apoptosis in ATRA-treated breast cancer cells. In our investigations bcl-2 expression was unaffected. It has not been demonstrated so far that the expression of the bcl-2 is directly regulated by ATRA. Probably, bcl-2 is being regulated by the dephosphorylating serine/threonine protein phosphatases. The bcl-2 phosphorylation after ATRA is a post-translational modification which is associated with a shortened half-life and may contribute to a change in its function, perhaps through degradation (49).

The decreased PP2A activity can be explained by the PP2A β regulatory subunit up-regulation. Our group showed that the B subunits exert an inhibitory effect on the catalytic subunit (18).

We believe that the underlying mechanisms of ATRA-induced cytotoxicity and apoptosis will open new therapeutic windows in breast cancer therapy. ATRA, in conjunction with hormonal/chemotherapeutic agents and/or serine/threonine protein phosphatase inhibitors may be utilized in combination therapy.

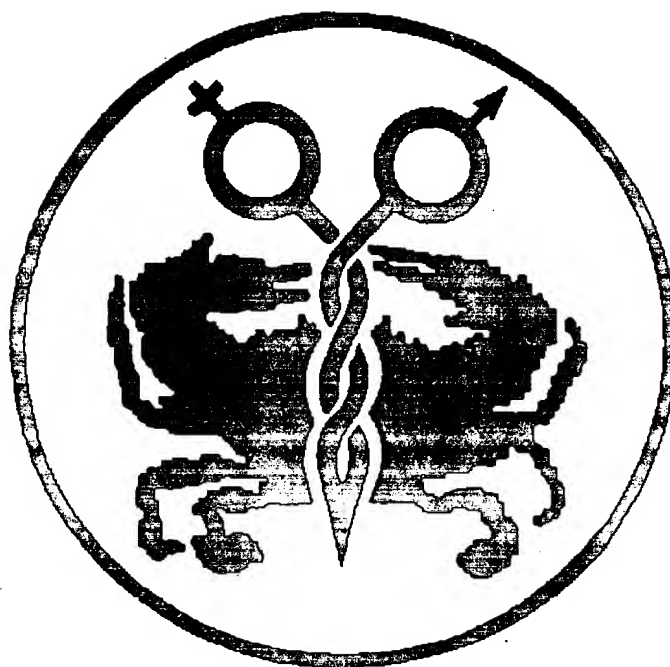
References

- Warrell RP, Frankel SR, Miller WH Jr, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A and Jakubowski A: Differentiation therapy of acute promyelocytic leukemia with retinoin (all-trans-retinoic acid). *N Engl J Med* 324: 1385-1393, 1991.
- Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R and Peters LJ: Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N Engl J Med* 323: 795-801, 1990.
- Lippman SM, Parkinson DR, Itri LM, Weber RS, Schantz SP, Ota DM, Schusterman MA, Krakoff IH, Gutterman JU and Hong WK: 13-cis-retinoic acid and interferon alpha-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. *J Natl Cancer Inst* 84: 235-241, 1991.
- Gudas LJ, Sporn MB and Roberts AB: Cellular biology and biochemistry of the retinoids. In: *The Retinoids*. Sporn MB, Roberts AB, Goodman DS (eds). Raven Press, New York, pp443-520, 1994.
- Benbrook D, Lernhardt E and Pfahl M: A new retinoic acid receptor identified from a hepatocellular carcinoma. *Nature* 333: 624-629, 1988.
- Brand N, Petkovich M, Krust A, Chambon P, De The H, Marchio A, Tiollais P and Dejean A: Identification of a second human retinoic acid receptor. *Nature* 332: 850-853, 1988.
- Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen JY, Staub A, Garnier JM and Mader S: Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* 68: 377-395, 1992.
- Mangelsdorf DJ, Borgmeyer U, Heyman RA, Zhou JY, Ong ES, Oro AE, Kakizuka A and Evans RM: Characterization of three RXR genes that mediate the action of 9-cis retinoic acid. *Genes Dev* 6: 329-344, 1992.
- Evans RM: The steroid and thyroid hormone receptor family. *Science* 240: 889-895, 1988.
- Green S and Chambon P: Nuclear receptors enhance our understanding of transcription regulation. *Trends Genet* 4: 309-314, 1988.
- Rishi AK, Shao ZM, Baumann RG, Li XS, Sheikh MS, Kimura S, Bashirelahi N and Fontana JA: Estradiol regulation of the human retinoic acid receptor alpha gene in human breast carcinoma cells is mediated via an imperfect half-palindromic estrogen response element and Sp1 motifs. *Cancer Res* 55: 4999-5006, 1995.

12. Roman SD, Ormandy CJ, Manning DL, Blamey RW, Nicholson RI, Sutherland RL and Clarke CL: Estradiol induction of retinoic acid receptors in human breast cancer cells. *Cancer Res* 53: 5940-5945, 1993.
13. Fitzgerald P, Teng M, Chandraratna RA, Heyman RA and Allegretto EA: Retinoic acid receptor alpha expression correlates with retinoid-induced growth inhibition of human breast cancer cells regardless of estrogen receptor status. *Cancer Res* 57: 2642-2650, 1997.
14. Rock CL, Saxe GA, Ruffin MT, August DA and Schottenfeld D: Carotenoids, vitamin A, and estrogen receptor status in breast cancer. *Nutr Cancer* 25: 281-296, 1996.
15. Stoll BA: Breast cancer: further metabolic-endocrine risk markers? *Br J Cancer* 76: 1652-1654, 1997.
16. Fontana JA, Miranda D and Mezu AB: Retinoic acid inhibition of human breast carcinoma proliferation is accompanied by inhibition of the synthesis of a Mr 39,000 protein. *Cancer Res* 50: 1977-1982, 1990.
17. Burg B, Leede BM, Kwakkenbos-Isbrucker L, Salverda S, De Laat SW and Saag PT: Retinoic acid resistance of estradiol-independent breast cancer cells coincides with diminished retinoic acid receptor function. *Mol Cell Endocrinol* 91: 149-157, 1993.
18. Nishikawa M, Omay SB, Toyoda H, Tawara I, Shima H, Nagao M, Hemmings BA, Mumby MC and Deguchi K: Expression of the catalytic and regulatory subunits of protein phosphatase type 2A may be differentially modulated during retinoic acid-induced granulocytic differentiation of HL-60 cells. *Cancer Res* 54: 4879-4884, 1994.
19. Mumby MC and Walter G: Protein serine/threonine phosphatases: structure, regulation, and functions in cell growth. *Physiol Rev* 73: 673-699, 1993.
20. Wera S and Hemmings BA: Serine/threonine protein phosphatases. *Biochem J* 311: 17-29, 1995.
21. Cohen P: The structure and regulation of protein phosphatases. *Ann Rev Biochem* 58: 453-508, 1989.
22. Encio IJ and Detera-Wadleigh SD: The genomic structure of the human glucocorticoid receptor. *J Biol Chem* 266: 7182-7188, 1991.
23. Klee CB and Cohen P: The calmodulin-regulated protein phosphatases. In: *Molecular Aspects of Cellular Regulation 'Calmodulin'*. Cohen P, Klee CB (eds). Elsevier, Amsterdam, pp225-228, 1988.
24. Liu J, Albers MW, Wandless TL, Luan S, Alberg DG, Belshaw PJ, Cohen P, MacKintosh C, Klee CB and Schreiber SL: Inhibition of T cell signalling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. *Biochemistry* 31: 3896-3899, 1992.
25. Milan D, Griffith J, Su M, Price ER and McKeon F: The latch region of calcineurin B is involved in both immunosuppressant-immunophilin complex docking and phosphatase activation. *Cell* 79: 437-439, 1994.
26. Flanagan WM, Cortesy B, Bram RJ and Crabtree GR: Nuclear association of a T-cell transcription factor blocked by FK-506 and cyclosporin A. *Nature* 352: 803-808, 1991.
27. Lu KP and Means AR: Regulation of the cell cycle by calcium and calmodulin. *Endocr Rev* 14: 40-44, 1993.
28. Rasmussen C, Garen C, Brining S, Kincaid RL, Means RL and Means AR: The calmodulin dependent protein phosphatase catalytic subunit (calcineurin A) is an essential gene in *Aspergillus nidulans*. *EMBO J* 13: 2545-2547, 1994.
29. Cunningham KW and Flink GR: Calcineurin-dependent growth control in *Saccharomyces Cerevisiae* mutant lacking PMC1, a homolog of plasma membrane Ca^{2+} ATPases. *J Cell Biol* 124: 351-353, 1994.
30. McGowan CH and Cohen P: Identification of two isoenzymes of protein phosphatase 2C in both rabbit skeletal muscle and liver. *Eur J Biochem* 166: 713-721, 1987.
31. McGowan CH, Campbell DG and Cohen P: Primary structure analysis proves that protein phosphatases 2C1 and 2C2 are isozymes. *Biochim Biophys Acta* 930: 279-282, 1987.
32. Wenk J, Trompeter HI, Pettrich KG, Cohen PT, Campbell DG and Mieskes G: Molecular cloning and primary structure of a protein phosphatase 2C isoform. *FEBS Lett* 297: 135-138, 1992.
33. Ohishi S, Endo M, Kobayashi T, Terasawa T, Murakami T, Onoda M, Ohnishi M, Itoh T, Tsuiji S and Tamura S: Enhanced expression of type 2C protein phosphatase gene during myogenic differentiation of C3H10T1/2 cells. *Biochem Int* 28: 345-351, 1992.
34. Brewis ND, Street AJ, Prescott AR and Cohen PT: PPX, a novel protein serine/threonine phosphatase localized to centrosomes. *EMBO J* 12: 987-996, 1993.
35. Hansen MB, Nielsen SE and Berg K: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods* 119: 203-210, 1989.
36. Warburton S and James R: Haemocytometer cell counts and viability studies. In: *Cell and Tissue Culture Laboratory Procedures*. Doyle A, Griffiths JB, Newell DG (eds). John Wiley, Chichester, pp411-415, 1994.
37. Aragene Y, Kulms D, Metze D, Wilkes G, Poppelman B, Luger TA and Schwarz T: Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/Apo-1) independently of its ligand CD95L. *J Cell Biol* 140: 171-182, 1998.
38. Lowry OH, Rosebrough NJ, Farr AL, *et al*: Protein measurement with the Folin-phenol reagent. *J Biol Chem* 193: 265-275, 1951.
39. Butler WB and Fontana JA: Responses to retinoic acid of tamoxifen-sensitive and -resistant sublines of human breast cancer cell line MCF-7. *Cancer Res* 52: 6164-6167, 1992.
40. Toma S, Isnardi L, Raffo P, Dastoli G, De Francisci E, Riccardi L, Palumbo R and Bollag W: Effects of all-trans-retinoic acid and 13-cis-retinoic acid on breast-cancer cell lines: growth inhibition and apoptosis induction. *Int J Cancer* 70: 619-627, 1997.
41. Mangiarotti R, Danova M, Alberici R and Pellicciari C: All-trans retinoic acid (ATRA)-induced apoptosis is preceded by G1 arrest in human MCF-7 breast cancer cells. *Br J Cancer* 77: 186-191, 1998.
42. Van heusden J, Wouters W, Ramaekers FC, Krekels MD, Dillen L, Borgers M and Smets G: All-trans-retinoic acid metabolites significantly inhibit the proliferation of MCF-7 human breast cancer cells *in vitro*. *Br J Cancer* 77: 26-32, 1998.
43. Hu Z-B, Minden MD and McCulloch EA: Post-transcriptional regulation of bcl-2 in acute myeloblastic leukemia: significance for response to chemotherapy. *Leukemia* 10: 410-416, 1996.
44. Bradbury DA, Aldington S, Zhu YM and Russell NH: Down-regulation of bcl-2 in AML blasts by all-trans retinoic acid and its relationship to CD34 antigen expression. *Br J Haematol* 94: 671-675, 1996.
45. Tawara I, Nishikawa M, Morita K, Kobayashi K, Toyoda H, Omay SB, Shima H, Nagao M, Kuno T and Tanaka C: Down-regulation by retinoic acid of the catalytic subunit of protein phosphatase type 2A during granulocytic differentiation of HL-60 cells. *FEBS Lett* 321: 224-228, 1993.
46. Omay SB, Nishikawa M, Morita K, Toyoda H, Nakai K, Shima H, Nagao M, Kitagawa S, Saito M and Shiku H: Decreased expression of protein phosphatase type 2A in HL-60 variant (HL-60AR) cells resistant to induction of cell differentiation by all-trans retinoic acid. *Exp Hematol* 23: 244-251, 1995.
47. Ratnasinghe D, Phang JM and Yeh GC: Differential expression and activity of phosphatases and protein kinases in adriamycin sensitive and resistant human breast cancer MCF-7 cells. *Int J Oncol* 13: 79-84, 1998.
48. Gopalakrishna R, Gundimeda U, Fontana JA and Clarke R: Differential distribution of protein phosphatase 2A in human breast carcinoma cell lines and its relation to estrogen receptor status. *Cancer Lett* 136: 143-151, 1999.
49. Hu ZB, Minden MD and McCulloch ED: Phosphorylation of bcl-2 after exposure of human leukemic cells to retinoic acid. *Blood* 5: 1768-1775, 1998.

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mRNA Levels of Catalytic Subunits of Protein Phosphatases 1, 2A, and 2C in Hepatocarcinogenesis

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The mRNA levels of three phosphoserine/phosphothreonine protein phosphatases, PP1, PP2A and PP2C, in rat liver have been determined by Northern blot analysis in various stages of rat chemical hepatocarcinogenesis using a Solt-Farber model. Five weeks after administration of diethylnitrosamine, the mRNA levels of PP1_α, PP2A and PP2C were elevated 8, 29 and 11 times, respectively, as compared to those of the control livers. However, in primary hepatoma induced according to the Solt-Farber model, the mRNA levels of all three protein phosphatases were dramatically decreased to normal levels or even to much lower levels, whereas the mRNA level of glutathione S-transferase placental form, a tumor marker protein, was greatly elevated as compared with that of the control livers. In a poorly differentiated hepatoma AH13, a line of rat ascites hepatoma, the mRNA level of PP1_α was 5.6 times higher than that of the control livers, whereas the mRNA level of PP2C was almost the same as that of the control livers and the level of PP2A mRNA was distinctly lower than that of the control livers. These data appear to suggest some involvement of protein phosphatases in hepatocarcinogenesis.

Key words: Protein phosphatase — Gene expression — Northern blot analysis — Rat liver — Hepatoma cell

Reversible phosphorylation of protein has now been recognized as a major regulatory mechanism of various cellular functions.¹⁻³ The fact that most oncogene products have protein kinase activity strongly suggests that cell growth is also controlled by phosphorylation/dephosphorylation of target proteins.^{4,5} In the past several years, our knowledge of protein phosphatases has enormously increased. At present, four principal phosphoserine/phosphothreonine protein phosphatases, termed PP1, PP2A, PP2B and PP2C, are known.¹ The nucleotide sequences of the cDNAs of these molecular species have been reported.⁶⁻¹¹ Recently, evidence suggesting important roles of protein phosphatases in growth control of cells has been accumulating, although the mechanisms involved are not known in any detail. Suganuma *et al.* reported that okadaic acid, a potent inhibitor of PP1 and PP2A, has strong promoter action in skin carcinogenesis.¹² In contrast, Sakai *et al.* reported flat reversion by okadaic acid of *raf* and *ret-II* transformants.¹³ Mutations of PP1 gene induce abnormal cell division.¹⁴⁻¹⁶ PP2A was reported to exist as complexes with viral oncogene proteins.¹⁷

We previously reported the separation, purification and characterization of protein phosphatases from rat livers, and neoplastic alterations of the phosphatase activities in rat hepatoma cells.¹⁸⁻²¹ Also, we showed that PP2C was replaced by the novel phosphatase H and that the enzyme activity of a divalent cation-inhibited protein phosphatase termed phosphatase N was tremendously increased in AH13.^{22,23} On the basis of their nature and properties, phosphatases H and N are supposed to belong to the PP2C and PP1 types, respectively. Recently, we reported the nucleotide sequence of the PP1_α cDNA from rat kidney, and a remarkable elevation in the PP1_α mRNA level in AH13.¹⁰ To elucidate the physiological significance of the neoplastic alterations of protein phosphatase, we have analyzed by Northern blot analysis the expression of the mRNAs of protein phosphatases PP1, PP2A and PP2C during hepatocarcinogenesis and in AH13. In the present paper, the significance of these results is discussed in relation to hepatocarcinogenesis.

MATERIALS AND METHODS

Animals, tissues, and hepatomas Male Sprague-Dawley rats (150-200 g) were subjected to a carcinogenic treat-

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ment using a Solt-Farber model.^{24,25} Animals were initially injected with DEN⁷ (200 mg/kg ip), followed by a diet containing 0.02% (w/v) AAF for 4 weeks, starting 2 weeks after administration of DEN, then subjected to partial hepatectomy 1 week after initiation of AAF. Primary hepatoma was also induced in male Sprague-Dawley rats (150–200 g) according to the Solt-Farber model. AH13, a line of rat hepatoma, was inoculated intraperitoneally into Donryu rats.²⁶ The hepatoma cells were harvested 5 days after inoculation, washed with physiological saline and stored at -80°C until use.

Poly(A)⁺ RNA preparations One gram of liver tissue was homogenized with a Polytron homogenizer (Kinematica, Switzerland) in a solution of 5 M guanidinium thiocyanate, 50 mM Tris-HCl, pH 7.5, 25 mM EDTA and 0.1 M 2-mercaptoethanol (soln. A). Cold ethanol (0.3 volume) was added to the homogenate, and the mixture was centrifuged for 5 min at 10,000 rpm at -10°C . The pellet was suspended in soln. A, homogenized and centrifuged for 3 min at 10,000 rpm at 4°C . After ethanol precipitation, the RNA preparations were suspended in 6 M guanidine hydrochloride, 25 mM EDTA and 0.1 M 2-mercaptoethanol, and precipitated with ethanol. The precipitates were dissolved in water, applied to an oligo(dT) cellulose column, washed with washing buffer (10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.1% SDS, 0.1 M NaCl), and then eluted with elution buffer (10 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.02% SDS). The eluates were used as poly(A)⁺ RNA fraction for Northern blot analysis.

cDNA probes The *Pst*I-*Sma*I fragment of 600 bp, the *Bst*EII-*Bgl*II fragment of 410 bp, and the *Pst*I-*Sal*I fragment of 550 bp were prepared from cDNAs of PP1_α, PP2A_α and PP2C₁, respectively.^{8–10} The full-length GST-P cDNA was a generous gift from Dr. Masaharu Sakai (Hokkaido University) and Dr. Masami Muramatsu (University of Tokyo).^{25,27,28} The DNA fragments were labeled with [α -³²P]dCTP by using the Multiprime DNA Labeling Kit (Amersham International plc, Buckinghamshire, England) and used for Northern blot analysis as probes.

Northern blot analysis of RNA Northern blot analysis was performed as described elsewhere.¹⁰ Five μg of poly(A)⁺ RNA was electrophoresed on 1.2% agarose gel containing 6% formaldehyde. RNA was transferred to a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). After being baked at 80°C for 2 h under vacuum, the filters were prehybridized in 5×SSPE (1×

SSPE; 1.8 M NaCl, 0.01 M NaH₂PO₄, 0.1 mM EDTA), 5×Denhardt's solution, 100 $\mu\text{g}/\text{ml}$ denatured DNA and 50% formamide overnight at 42°C . Hybridization was performed in the same solution with the multiprime radioactive cDNA probes described above overnight at 42°C . Filters were then washed twice in 5×SSPE at room temperature for 10 min, followed by washing at 50°C for 30 min in 5×SSPE containing 0.1% SDS, followed by 0.1×SSPE containing 0.1% SDS at 65°C for 2 h. Filters were exposed to XRP-5 X-ray film (Eastman Kodak Company, Rochester, NY) at -80°C with intensifying screens. The density was scanned with a densitometer (Shimadzu dual-wavelength TLC scanner). The filters were reused several times after the probes had been removed by washing the filters in 0.1×Denhardt's solution containing 2 mM EDTA and 5 mM Tris-HCl, pH 8.0, at 70°C for 2 h.

Chemicals Guanidinium thiocyanate and guanidine hydrochloride were purchased from Fluka Chemie AG (Buchs, Switzerland) and Wako Pure Chemical In-

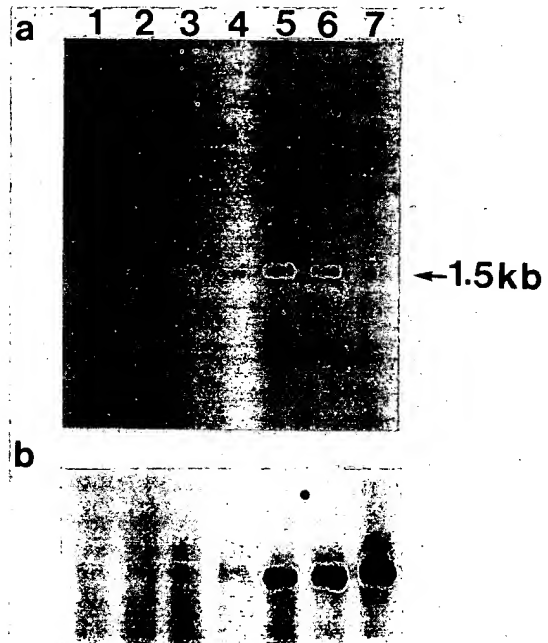


Fig. 1. Expression of PP1 mRNA in hepatocarcinogenesis. Five μg samples of poly(A)⁺ RNA in livers at various stages of chemical carcinogenesis according to the protocol of the Solt-Farber model and in a primary hepatoma induced according to the same protocol were subjected to Northern blot analysis using a) *Pst*I-*Sma*I fragment of PP1_α cDNA and b) GST-P cDNA as probes, respectively. Other conditions are described in the text. Lane 1, control; lanes 2–6, 2, 3, 4, 5, and 6 weeks after administration of DEN, respectively; lane 7, primary hepatoma induced according to the Solt-Farber model.

⁷ Abbreviations used in this paper: DEN, diethylnitrosamine; AAF, 2-acetylaminofluorene; MeDAB, 3'-methyl-4-dimethylaminoazobenzene; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; GST-P, glutathione S-transferase placental form.

dustries. Ltd. (Osaka), respectively. [α - 32 P]dCTP and oligo(dT) cellulose were purchased from DuPont/NEN Research Products (Boston, MA) and Pharmacia LKB Biotechnology AB (Uppsala, Sweden), respectively.

RESULTS

Expression levels of PP1 mRNA Expression levels of PP1 mRNA were analyzed by Northern blot analysis in livers at various stages of chemical hepatocarcinogenesis according to the Solt-Farber protocol and in a primary hepatoma induced according to a Solt-Farber model. As shown in Fig. 1, a single band of 1.5 kb was detected in all samples employed, corresponding to mRNA of PP1 α .¹⁰ After administration of DEN, the mRNA level gradually increased, reached the maximum 5 weeks after administration (8 times higher than that of control livers) and then began to decline. In primary hepatoma induced according to a Solt-Farber model, the mRNA level was similar to the control level. Under identical conditions, the expression of GST-P mRNA was remarkably elevated as previously reported.²⁵⁾

Expression levels of PP2A mRNA The mRNA levels of PP2A were determined under identical conditions of

Northern blot analysis except that the probe for PP2A was used. As already reported, two mRNA bands (a major one of 2.0 kb and a minor one of 2.7 kb) were detected.⁸⁾ As shown in Fig. 2, the major band increased during hepatocarcinogenesis, being 29 times higher at 5 weeks after administration of DEN than that of the control livers. In the primary hepatoma, the mRNA level of PP2A was similar to the control level.

Expression levels of PP2C mRNA Figure 3 shows the results of Northern blot analysis of PP2C. With PP2C, a similar gene expression pattern to those of PP1 and PP2A was observed during hepatocarcinogenesis. Five weeks after administration of DEN, the mRNA level of PP2C was 11 times higher than that of the control rats. In the primary hepatoma, the PP2C mRNA level was lower than that of the control livers.

mRNA levels of protein phosphatases in AH13 In accordance with our previous results,¹⁰⁾ the mRNA level of PP1 α in AH13 was 5.6 times higher than that of the control livers (Fig. 4). As the primary hepatoma induced by MeDAB showed a slight increase in the gene expression as compared to the control livers (not shown), these results strongly suggest an enhancement of the gene expression of PP1 α during progression of hepatocarcino-

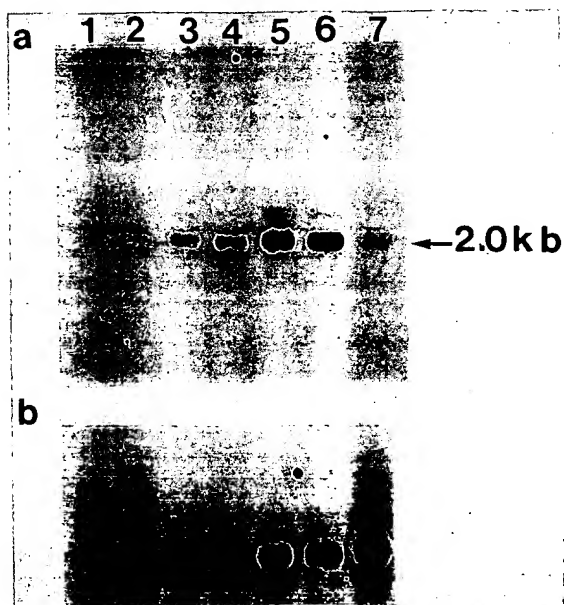


Fig. 2. Expression of PP2A mRNA in hepatocarcinogenesis. Experimental conditions in a) and b) were essentially identical to those in Fig. 1 except that *Pst*I-*Sma*I fragment of PP1 α cDNA was replaced by *Bst*EII-*Bgl*II fragment of PP2A α cDNA. Lane 1, control; lanes 2-6, 2, 3, 4, 5, and 6 weeks after administration of DEN, respectively; lane 7, primary hepatoma induced according to the Solt-Farber model.

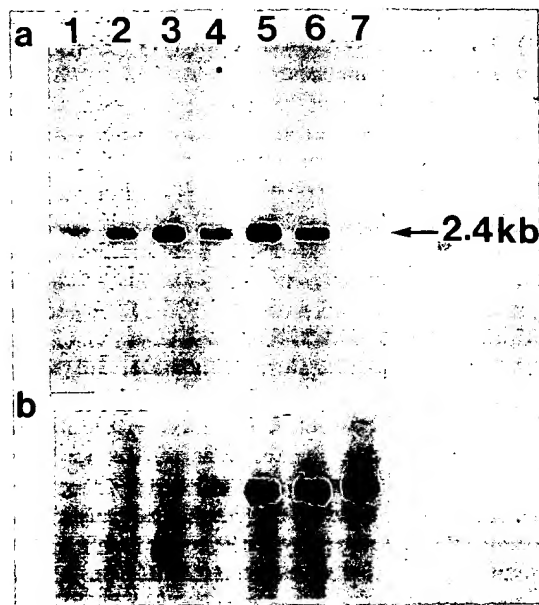


Fig. 3. Expression of PP2C mRNA in hepatocarcinogenesis. Experimental conditions in a) and b) were essentially identical to those in Fig. 1 except that the *Pst*I-*Sma*I fragment of PP1 α cDNA was replaced by the *Pst*I-*Sal*I fragment of PP2C cDNA. Lane 1, control; lanes 2-6, 2, 3, 4, 5, and 6 weeks after administration of DEN, respectively; lane 7, primary hepatoma induced according to the Solt-Farber model.

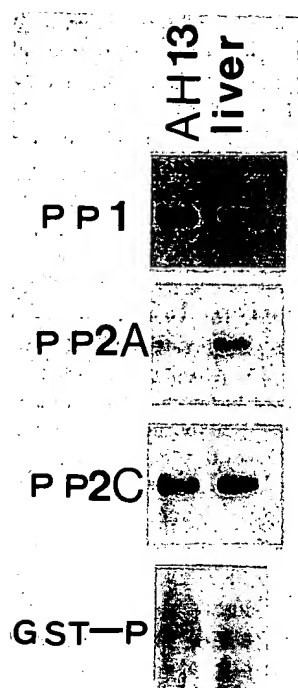


Fig. 4. Expression of PP1, PP2A and PP2C mRNAs in AH13 and in liver. Five μ g samples of poly (A)⁺ RNAs from AH13 hepatoma cells and control rat livers were subjected to Northern blot analysis. Probes used for PP1 and GST-P, PP2A, and PP2C were identical to those described in the legends to Fig. 1, Fig. 2, and Fig. 3, respectively. Other conditions are described in the text.

genesis. In striking contrast to PP1 α , the gene expression of PP2A was much lower in AH13 than the control livers, whereas there was no alteration in the mRNA level of PP2C.

DISCUSSION

The present study demonstrates that expression levels of mRNAs of protein phosphatases PP1 α , PP2A and PP2C were remarkably elevated in livers at preneoplastic stages of hepatocarcinogenesis, but was decreased in primary hepatoma to below the control level. In AH13, a poorly differentiated hepatoma line, the mRNA level of PP1 α was greatly and selectively elevated as compared with that of the control livers, whereas the mRNA levels of PP2A and PP2C in AH13 were decreased and unchanged from the control values, respectively.

Two weeks after administration of DEN, altered foci appeared in the livers. But, upon administration of AAF at this stage, the foci mostly disappeared because of the toxicity of AAF. Thereafter the area of hyperplastic

nodules gradually increased. The ratios of area of hyperplastic nodule cells to area of normal liver cells at 4, 5, and 6 weeks after administration of DEN were 0.05–0.10, 0.25–0.30, and 0.42–0.54, respectively.²⁵⁾ The mRNA levels were significantly increased 3 weeks after administration of DEN. At this stage, hyperplastic nodules were very small, and the mRNA levels of GST-P were also very low. Then the phosphatase mRNA levels further increased, but the patterns of this increase varied from experiment to experiment. In most cases, the mRNA levels at 4 weeks after administration of DEN were slightly lower than those at 3 weeks and 5 weeks after the administration as shown in Figs. 1–3. However, in some cases, the mRNA levels at 4 weeks after the administration were intermediate between those at 3 weeks and 5 weeks after the administration. So we have presented what we regard as typical data. We speculate that the increase at 3 weeks after the administration of DEN might be ascribed to the response of normal liver cells to DEN, the levels being transiently decreased 4 weeks after the administration probably because of the toxicity of AAF to liver cells, and then increased because of the formation of hyperplastic nodules. So the levels at 4 weeks after the administration seem to reflect the influences of at least two factors, i.e., response to DEN and formation of hyperplastic nodules.

The decreases of the mRNA levels in primary hepatoma were unexpected, because previous studies revealed elevation of the mRNA levels of PP1 and PP2A in hepatoma cells.^{8,10)} In the primary hepatoma presented here, in which the mRNA levels of protein phosphatases were all decreased, the mRNA level of GST-P, a tumor marker protein, was dramatically elevated, as already reported.^{25,27,28)} These results exclude the possibility that the decrease in the mRNA levels in the primary hepatoma was due to artifacts during preparation of the RNAs used. Increase in protein kinase activities during hepatocarcinogenesis and in hepatomas has been reported.^{29,30)} We have also reported an increase in casein kinase 1 activity in AH13.²⁶⁾ Moreover, Beer *et al.* reported the elevation in rat hepatoma cells of mRNA for *c-raf*, which encodes a protein kinase catalyzing the phosphorylation of serine and threonine.³¹⁾ Also, Maridor *et al.* reported that expressions of mRNA for all three subunits of casein kinase II were high in early embryos and decreased substantially during embryogenesis, suggesting involvement of this protein kinase in control of cell proliferation.³²⁾ Persons *et al.* reported that transfection of NIH 3T3 fibroblasts with protein kinase C enhanced tumorigenicity via enhancement of the mRNA levels of the PKC-I.³³⁾ These results, taken together, suggest that the mRNA levels of protein kinases might be predominant, at least in primary hepatomas, over those of protein phosphatases.

Recently, evidence demonstrating involvement of both phosphorylation and dephosphorylation in the cell cycle has been accumulating.³⁴⁻³⁶ Taking account of these facts, our present results appear to show an important role of protein phosphatases in the regulation of cell growth at preneoplastic stages of hepatocarcinogenesis. It is of great interest that there was a striking difference in gene expressions of protein phosphatases among livers at preneoplastic stages, primary hepatoma, and transplanted, poorly differentiated hepatoma AH13. Since not only PP2A but also PP1 can form heterooligomers and their activities are subject to multiple control mechanisms, these alterations in mRNA levels might not necessarily reflect the protein phosphatase activities. But these features of the gene expression may represent a new control mechanism of protein phosphatase. It is tempting to suggest a relationship of the elevation of PP1_α mRNA levels to malignancy during the progression of hepatocarcinogenesis.

We previously reported a decrease in the enzyme activity of PP2C and emergence of the novel protein phosphatase H.²² We also found in AH13 a marked

increase in protein phosphatase N belonging to the PP1 category.²³ These previous results may be reflections of the increase in gene expression of PP1 presented in this paper. Attempts to elucidate the relationship between those enzymatic alterations previously reported and the present data on the mRNA levels are in progress.

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REFERENCES

- 1) Cohen, P. The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.*, **58**, 453-508 (1989).
- 2) Hunter, T. and Cooper, A. J. Protein-tyrosine kinases. *Ann. Rev. Biochem.*, **54**, 897-930 (1985).
- 3) Rosen, O. After insulin binds. *Science*, **237**, 1452-1458 (1987).
- 4) Yarden, Y. and Ullrich, A. Growth factor receptor tyrosine kinases. *Ann. Rev. Biochem.*, **57**, 443-478 (1988).
- 5) Bishop, M. J. Cellular oncogenes and retroviruses. *Ann. Rev. Biochem.*, **52**, 301-354 (1983).
- 6) Cohen, P. and Cohen, P. T. W. Protein phosphatases come of age. *J. Biol. Chem.*, **264**, 21435-21438 (1989).
- 7) Cohen, P. T. W., Brewis, D. N., Hughes, V. and Mann, D. J. Protein serine/threonine phosphatase; an expanding family. *FEBS Lett.*, **263**, 355-359 (1990).
- 8) Kitagawa, Y., Tahira, T., Ikeda, I., Kikuchi, K., Tsuiki, S., Sugimura, T. and Nagao, M. Molecular cloning of cDNA for the catalytic subunit of rat liver type 2A protein phosphatase, and detection of high levels of expression of the gene in normal and cancer cells. *Biochim. Biophys. Acta*, **951**, 123-129 (1988).
- 9) Tamura, S., Lynch, K. R., Larner, J., Fox, J., Yasui, A., Kikuchi, K., Suzuki, Y. and Tsuiki, S. Molecular cloning of rat type 2C(IA) protein phosphatase mRNA. *Proc. Natl. Acad. Sci. USA*, **86**, 1796-1800 (1989).
- 10) Kitamura, K., Mizuno, Y., Sasaki, A., Yasui, A., Tsuiki, S. and Kikuchi, K. Molecular cloning and sequence analysis of cDNA for the catalytic subunit 1_α of rat kidney type 1 protein phosphatase, and detection of the gene expression at high levels in hepatoma cells and regenerating livers as compared to rat livers. *J. Biochem.*, **109**, 307-310 (1991).
- 11) Sasaki, K., Shima, H., Kitagawa, Y., Irino, S., Sugimura, T. and Nagao, M. Identification of members of the protein phosphatase 1 gene family in the rat and enhanced expression of protein phosphatase 1_α gene in rat hepatocellular carcinomas. *Jpn. J. Cancer Res.*, **81**, 1272-1280 (1990).
- 12) Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, S., Ojika, M., Wakamatsu, K., Yamada, K. and Sugimura, T. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proc. Natl. Acad. Sci. USA*, **85**, 1768-1771 (1988).
- 13) Sakai, R., Ikeda, I., Kitani, H., Fujiki, H., Takaku, F., Rapp, U., Sugimura, T. and Nagao, M. Flat reversion by okadaic acid of *raf* and *ret-II* transformants. *Proc. Natl. Acad. Sci. USA*, **86**, 9946-9950 (1989).
- 14) Booher, R. and Beach, D. Involvement of a type 1 protein phosphatase encoded by *bws1*⁺ in fission yeast mitotic control. *Cell*, **57**, 1009-1016 (1989).
- 15) Doonan, J. H. and Morris, N. R. The *bimG* gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase 1. *Cell*, **57**, 987-996 (1989).
- 16) Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T. and Yanagida, M. The fission yeast *dis2*⁺ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. *Cell*, **57**, 997-1007 (1989).
- 17) Pallas, C. D., Shahrik, K. L., Martin, B. L., Jaspers, S., Miller, T. B., Brautigan, D. L. and Roberts, T. M. Poly-

- oma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. *Cell*, **60**, 167-176 (1990).
- 18) Kikuchi, K., Tamura, S., Hiraga, A. and Tsuike, S. Glycogen synthase phosphatase of rat liver. Its separation from phosphorylase phosphatase on DE-52 columns. *Biochem. Biophys. Res. Commun.*, **75**, 29-37 (1977).
 - 19) Tamura, S., Kikuchi, H., Kikuchi, K., Hiraga, A. and Tsuike, S. Purification and subunit structure of a high-molecular-weight phosphoprotein phosphatase (phosphatase II) from rat liver. *Eur. J. Biochem.*, **104**, 347-355 (1980).
 - 20) Tamura, S. and Tsuike, S. Purification and subunit structure of rat-liver phosphoprotein phosphatase, whose molecular weight is 260000 by gel filtration (phosphatase IB). *Eur. J. Biochem.*, **111**, 217-224 (1980).
 - 21) Hiraga, A., Kikuchi, K., Tamura, S. and Tsuike, S. Purification and characterization of Mg^{2+} -dependent glycogen synthase phosphatase (phosphoprotein phosphatase IA) from rat liver. *Eur. J. Biochem.*, **119**, 503-510 (1981).
 - 22) Kikuchi, K., Shineha, R., Hiraga, A., Tamura, S., Kikuchi, H. and Tsuike, S. Cytosolic protein phosphatases of rat ascites hepatoma AH-13 as compared with those of rat liver: isolation and characterization of a novel protein phosphatase. *Gann*, **75**, 388-394 (1984).
 - 23) Shineha, R., Kikuchi, K., Tamura, S., Hiraga, A., Suzuki, Y. and Tsuike, S. Particulate-associated protein phosphatases of rat hepatomas as compared with the enzymes of rat liver. *Jpn. J. Cancer Res.*, **81**, 161-168 (1990).
 - 24) Solt, D. and Farber, E. New principle for the analysis of chemical carcinogenesis. *Nature*, **263**, 701-703 (1976).
 - 25) Sato, K. Glutathione S-transferases and hepatocarcinogenesis. *Jpn. J. Cancer Res.*, **79**, 556-572 (1988).
 - 26) Kikuchi, K., Hirai, R., Mitsui, H., Kiuchi, Y. and Tsuike, S. Casein and histone kinases of a rat ascites hepatoma as compared with those of rat liver. *Jpn. J. Cancer Res.*, **76**, 1154-1161 (1985).
 - 27) Sugioka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. and Muramatsu, M. Cloning and the nucleotide sequence of rat glutathione-S-transferase P cDNA. *Nucleic Acids Res.*, **13**, 6049-6057 (1985).
 - 28) Sugioka, Y., Fujii-Kuriyama, Y., Kitagawa, T. and Muramatsu, M. Changes in polypeptide pattern of rat liver cells during chemical hepatocarcinogenesis. *Cancer Res.*, **45**, 365-378 (1985).
 - 29) Olson, W. J. Liver tyrosine kinase activation during early stage of chemical hepatocarcinogenesis. *J. Cell. Biochem.*, **27**, 175-180 (1985).
 - 30) Weber, A., Cottreau, D., Henry, J., Phan Dinh Tuy, F., Skala, H. and Kahn, A. Protein kinases of rat liver during hepatocarcinogenesis induced by an azo dye. *Eur. J. Biochem.*, **130**, 447-456 (1983).
 - 31) Beer, D. G., Neveu, M. J., Paul, D. L., Rapp, U. R. and Pitot, H. C. Expression of the c-*raf* protooncogene, γ -glutamyltranspeptidase, and gap junction protein in rat liver neoplasms. *Cancer Res.*, **48**, 1610-1617 (1988).
 - 32) Maridor, G., Park, W., Krek, W. and Nigg, E. A. Casein kinase II. *J. Biol. Chem.*, **266**, 2362-2368 (1991).
 - 33) Persons, D. A., Wilkison, W. O., Bell, R. M. and Finn, O. J. Altered growth regulation and enhanced tumorigenicity of NIH 3T3 fibroblasts transfected with protein kinase C-I cDNA. *Cell*, **52**, 447-458 (1988).
 - 34) Minshull, J., Golsteyn, R., Hill, S.C. and Hunt, T. The A- and B-type cyclin associated cdc2 kinases in *Xenopus* turn on and off at different times in the cell cycle. *EMBO J.*, **9**, 2865-2875 (1990).
 - 35) Roy, M. L., Singh, B., Gautier, J., Aringhaus, B. R., Nordeen, K. S. and Maller, L. J. The cyclin B2 component of MPF is a substrate for the c-*mos*⁺ proto-oncogene product. *Cell*, **61**, 825-831 (1990).
 - 36) Tamura, T., Simon, E., Geschwill, K. and Niemann, H. cdc2/pp56-62 are *in vitro* substrates for the tyrosine kinase encoded by the v-*fms* oncogene. *Oncogene*, **5**, 1259-1263 (1990).

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